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EXPRESS MAIL CERTIFICATE

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DARBY & DARBY P.C.
805 Third Avenue
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212-527-7700

Docker No: **6670/0H748**

Box PATENT APPLICATION
Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Enclosed please find an application for United States patent as identified below:

Inventor/s (name ALL inventors): **Pierre DESLONGCHAMPS; Yves DORY; Gilles BERTHIAUME; Luc OELLET; Ruoxi LAN**

Title: **COMBINATORIAL SYNTHESIS OF LIBRARIES OF MACROCYCLIC COMPOUNDS USEFUL IN DRUG DISCOVERY**

including the items indicated:

1. Specification and 23 claims: 9 indep.; 12 dep.; 2 multiple dep.

2. [] Executed declaration and power of attorney
   [X] Unexecuted declaration and power of attorney

3. [X] Formal drawings, 6 sheets (Figs. 1, 2a-2b, 3, 4a-4b)
   [] Informal drawings, _ sheets (Figs.)

4. [] Assignment for recording to:

5. [] Verified Statement Claiming Small Entity Status

6. [] Check in amount of $<u>.00</u>, (<u>$</u> filing; <u>$</u> recording)
   (See attached **Fee Computation Sheet**)

7. [] Preliminary Amendment

8. [] Information Disclosure Statement

9. [] Please amend the description by inserting the following paragraph after the line containing the title on page 1:
   "This patent application claims the priority of U.S. provisional patent application No. 60/, which is incorporated herein by reference."

Priority is claimed for this application, corresponding application/s having been filed as follows:

Country:	CANADA
Number:	2,284,459
Date:	OCTOBER 4, 1999

The priority documents [] are enclosed
[**X**] will follow.

Respectfully submitted,

Dated: October 4, 2000

Anne E. Zitron, Ph.D.
Reg. No. 41,391
Agent for Applicant(s)

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G KARASZI
Name (Print)

G Karaszi
Signature

Docket No.: 6670/0H748

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Pierre DESLONGCHAMPS et al.

Serial No.: TBA   Art Unit: TBA

Filed: Concurrently Herewith   Examiner: TBA

For: COMBINATORIAL SYNTHESIS OF LIBRARIES OF MACROCYCLIC COMPOUNDS USEFUL IN DRUG DISCOVERY

PRELIMINARY AMENDMENT

Hon. Commissioner of
Patents and Trademarks
Washington, DC 20231

Sir:

Prior to examination of the above-identified patent application please make the following amendments:

In The Claims

In claims 9 and 20, line 2 in each, please delete "and" and insert --or-- therefor.

REMARKS

Multiply dependent claims 9 and 20 have been amended to properly recite the claims from which the amended claims depend.

Prompt and favorable action is respectfully solicited.

Respectfully submitted,

Dated: October 4, 2000

Anne E. Zitron, Ph.D.
Reg. No. 41,391
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COMBINATORIAL SYNTHESIS OF LIBRARIES OF MACROCYCLIC COMPOUNDS USEFUL IN DRUG DISCOVERY

FIELD OF THE INVENTION

The present invention relates to new macrocyclic compounds of biologically interesting structures.

The invention also relates to a process for preparing these new compounds by lactam or Mitsunobu cyclization.

BACKGROUND OF THE INVENTION

As everybody knows, medicinal chemistry research has been dramatically transformed by biotechnology. Previously synthetic chemistry and natural products screening dominated drug research, but now molecular biology has become a driving force behind screening and the establishment of molecular targets.

Over the last years, most of the research in biotech companies has been directed to peptide and protein therapeutics in spite of problems associated with their low bioavailability, rapid metabolism, and lack of oral activity. Because of these limitations, research groups continue to rely upon chemical synthesis of nonpeptide substances for drug discovery, recognizing that small molecules are likely to remain the most viable avenue for the identification and optimization of potential drugs.

As is also known, combinatorial chemistry is a technique by which large chemical libraries can be generated by

connecting together appropriate chemical building blocks in a systematic way. The ability to generate such large, chemically diverse libraries, either in a combinatiorial fashion or by any other high throughput parallel synthetic methods, combined with high throughput screening techniques, provides an immensely powerful tool for drug lead discovery and optimization.

Drug companies are increasingly interested in harnessing the ability of combinatorial synthesis to produce large collections (or libraries) of molecules to augment their existing sources of molecular diversity, and to fully exploit their capacity to capture millions of biological assay data points annually using high throughput robotic screening instrumentation.

This new science is still in its infancy, and to date most successful scaffold are derived from small heterocycles which are usually synthesized in very few steps. Thus several focused libraries have been built around bioactive cores such as benzodiazepines. However, this approach cannot be considered as a true method to generate innovative lead structures. Rather, it is a mean to optimize existing leads and is usually applied in drug development schemes.

Random libraries destined to search for innovative leads are very few today. As one example, a library based on diketopiperazine yielded a new submicromolar lead for a neurokinin-2 receptor after screening of this library on a variety of targets.

It is obvious that not all scaffolds may lead to potent drug candidates. Some very simple molecules requiring only one or two chemical steps may seem very attractive due to the huge size of the libraries that can be generated from them. Nonetheless, too simple molecules do not usually provide useful leads since they tend to lack target specificity, a prerequisite for a molecule to become a drug.

A class of organic structures with outstanding pharmaceutical activity has been termed as "macrocycle family". Compounds like Taxol, Epothilone, Erythromycin, Neocarzinostatin, Rifampin and Amphotericin are either under clinical study or already marketed drugs and belong to this important family. Most of these products are of natural origin, since they are not usually tackled by medicinal chemists due to lack of knowledge associated with their synthesis.

Over the last years, the present inventors have developed expertise in the field of macrocycles synthesis. With such an expertise, they have developed a method of synthesis and evaluation of libraries of partially peptidic macrocycles which mimic β-turns, thereby making it possible to quickly explore huge quantities of conformationally restricted structures.

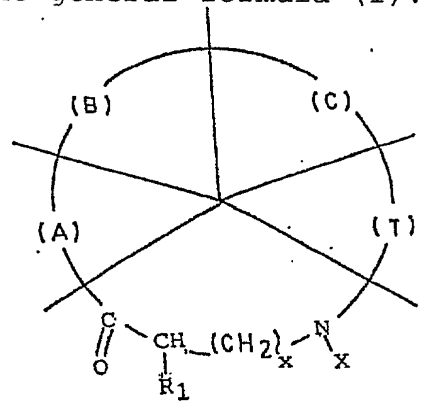
OBJECTS AND SUMMARY OF THE INVENTION

A first object of the present invention is to provide a process for preparing macrocyclic compounds, which process can be carried out with a large variety of functional

groups and in the presence of a large variety of solvent systems and resins, and thus can be used for preparing a large variety of macrocyclic compounds of biologically interesting structures that could be used for carrying out screening assays or as instrumental for the synthesis of other macrocyclic compounds. Libraries of such synthetic compounds should actually be as attractive as the libraries of extracted natural products which are presently used in high throughput biological screening assays.

Another object of the invention is to provide libraries of macrocyclic compounds incorporating two to five building units: one to four amino-acids and a tether chain for controlling the overall shape of the molecule.

More specifically, the macrocyclic compounds of the invention have the general formula (I):



(I)

- where part (A) is a $-\underset{\underset{O}{\|}}{C}-\underset{\underset{R_2}{|}}{CH}-(CH_2)_y-NH-$ bivalent radical

having its -NH- group linked to the carbonyl group of part $-\underset{\underset{O}{\|}}{C}-\underset{\underset{R_1}{|}}{CH}-(CH_2)_x-\underset{\underset{X}{|}}{N}-$, a $-(CH_2)_y-$ bivalent radical, or a covalent bonds;

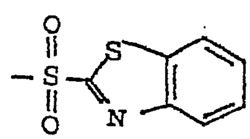
- where part(B) is a $-\underset{\underset{O}{\|}}{C}-\underset{\underset{R_3}{|}}{CH}-(CH_2)_z-NH-$ bivalent radical

having its -NH- group linked to part (A), a $-(CH_2)_z-$ bivalent radical, or a covalent bond;

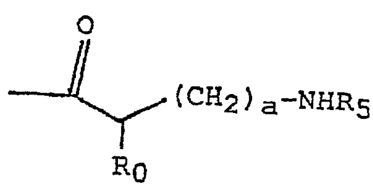
- where part (C) is a $-\underset{\underset{O}{\|}}{C}-\underset{\underset{R_4}{|}}{CH}-(CH_2)_t-NH-$ bivalent radical

having its -NH- group linked to part (B), a $-(CH2)_t-$ bivalent radical, or a covalent bond;

- where part (T) is a - Y - L - Z - radical; and
- where X is a monovalent group selected from the group consisting of: $-SO_2-Ar$, $-SO_2-CH_3$, $-SO_2-CF_3$, -H, -COH, $-CO-CH_3$, -CO-Ar, -CO-R, -CO-NHR, -CO-NHAr, -CO-O-tBu, $-CO-O-CH_2-Ar$



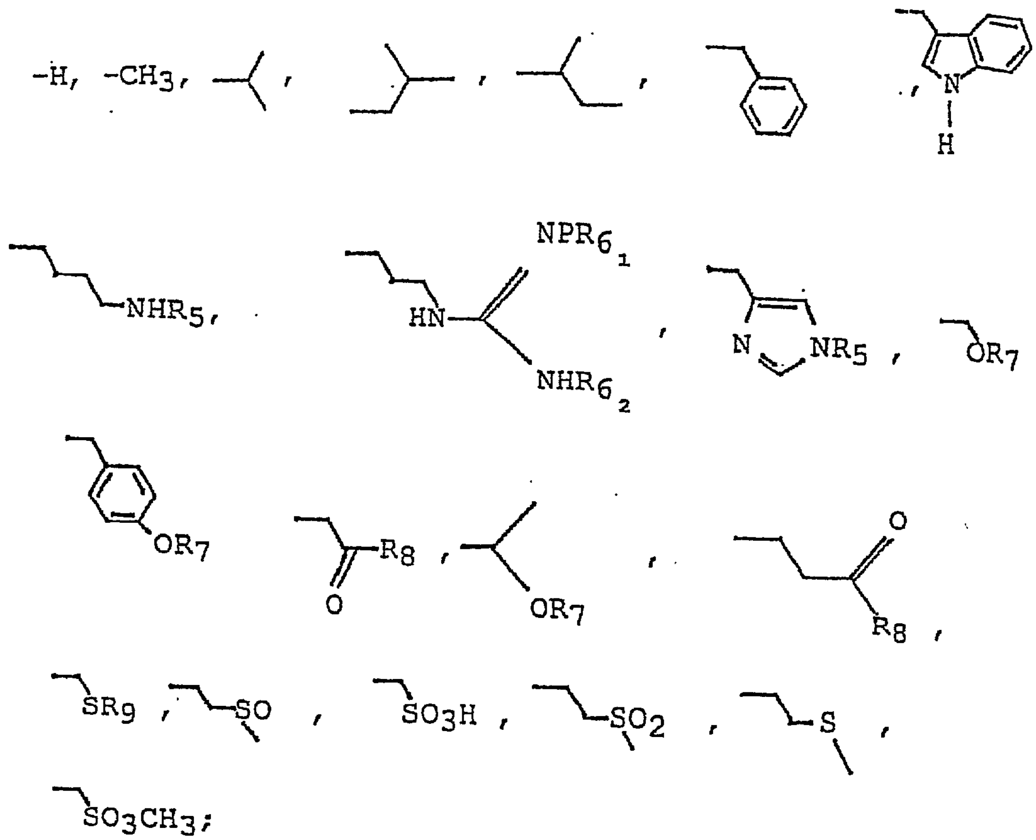
, and



,

- Ar being an aromatic group or substituted aromatic group,
- a being an integer selected from the group consisting of 0, 1 and 2,
- R being a monovalent group $-(CH_2)_n-CH_3$ or $-(CH_2)_n-Ar$ with n being an integer from 1 to 16,

- R_0, R_1, R_2, R_3 and R_4 being independently selected from the group consisting of:



R_5, $R6_1$ and $R6_2$ each being a monovalent radical independantly selected from the group consisting of: -H, $-SO_2-CH_3$, $-SO_2-CF_3$, -COH, $-COCH_3$, -CO-Ar, -CO-R or -CO-NHR wherein R is defined as above, -CONHAr, -COO-tBu and $-COO-CH_2-Ar$, said radical being or not substituted by at least one monovalent group selected in the group consisting of:

$-O-CH_3$, $-CH_3$, $-NO_2$, $-NH_2$, $-NH-CH_3$, $-N(CH_3)_2$, -CO-OH, $-CO-O-CH_3$, $-CO-CH_3$, $-CO-NH_2$, OH, F, Cl, Br and I;

R_7 being a monovalent radical selected from the group consisting of:

-H, -COH, -CO-CH_3, -CO-R wherein R is defined as above, -CO-Ar and -CO-tBu, said radical being substituted or not by at least one substituent selected from the group consisting of:

-O-CH_3, -CH_3, -NO_2, -NH_2, -NH-CH_3, -N$(CH_3)_2$, -CO-OH, -CO-O-CH_3, -CO-CH_3, -CO-NH_2, OH, F, Cl, Br and I;

R_8 being a monovalent radical selected from the group consisting of: -OH, -NH_2, -OCH_3, -$NHCH_3$, -O-tBu and -O-CH_2-Ar, said radical substituted or not by at least one group selected in the group consisting of:

-O-CH_3, -CH_3, -NO_2, -NH-CH_3, -N$(CH_3)_2$, -CO-OH, -CO-O-CH_3, -CO-CH_3, -CO-NH_2, OH, F, Cl, Br, I;

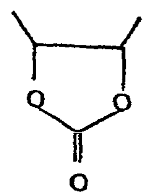
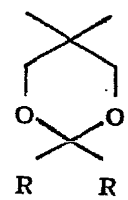
R_9 being a monovalent radical selected in the group consisting of: -H, -tBu, -CO-CH_3, -COAr, -CO-R wherein R is defined as above and -COH, said radicals substituted or not by at least one a monovalent group selected from the group consisting of:

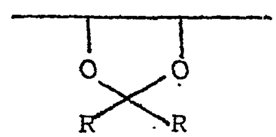
-O-CH_3, -CH_3, -NO_2, -NH-CH_3, -N$(CH_3)_2$, -CO-OH, -CO-O-CH_3, -CO-CH_3, -CO-NH_2, OH, F, Cl, Br and, I;

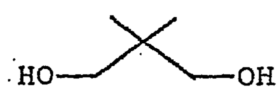
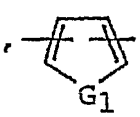
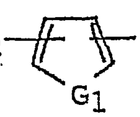
- where Y is a bivalent group -CH_2- or -CO-;
- where Z is a bivalent group -NH- or -O-;
- wherein x, y, z and t are integers each independently selected from the group consisting of 0,1 and 2;

- wherein L is a bivalent radical selected from the group consisting of:

  $-(CH_2)_d-A-(CH_2)_j-B-(CH_2)_e-$, d and e being independently an integer from 1 to 5, j being an integer from 0 to 5,
  with A and B being independently selected from the group consisting of:
  -O-, -NH-, -NR- wherein R is defined as above, -S-, -CO-, -SO-, -CO-O-, -O-CO-, -CO-NH-, -NH-CO-, $-SO_2-NH-$, $-NH-SO_2-$,

   , 

   , -CHOH-,  ,

   , -CH=CH- with the configuration Z or E, -C≡C-,  G_2 and $-G_2$ 

  with the substituent $-G_2-$ in a 1,2, 1,3 or 1,4 position,
  G_1 being selected from the group consisting of:
    -O-, -NH-, -NR- wherein R is defined as above, -S-, -CH=CH- with a Z configuration, and -CH=N-; and

G_2 being selected from the group consisting of:

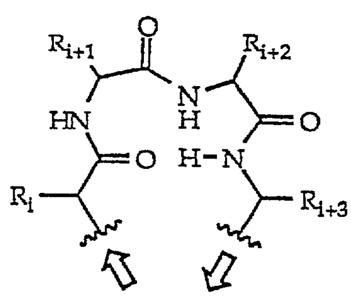
-O-, -NH-, -CO-, -NR- wherein R is defined as above, -CO-O-, -O-CO-, -CO-NH-, -NH-CO-, $-SO_2$-NH- and -NH-SO_2-.

Salts of said compounds are also within the scope of the invention.

As may be understood, the new macrocyclic compounds according to the invention incorporate two to five building units, one to four amino-acids units and a tether chain which controls the shape of the molecule.

These compounds display enhanced stability towards peptidases and exhibit facilitated cell penetration as compared to the corresponding open chain linear equivalents.

Some of the compounds according to the invention includes a β-turn motif within their rings:



β-turn

It is known that β-turn is one of the three major motifs of peptide and protein secondary structure. β-turn plays a key role in many biological molecular recognition events including interactions between antigens and antibodies, peptide hormones and their receptors, and regulatory enzymes and their corresponding substrates. In order to attain high affinity and selective binding to a targeted receptor, a β-turn mimetic must reproduce both the functionality and the orientation of the side chains of the receptor-bound peptide ligand.

The inherent diversity in β-turn structure compounded with difficulties in identifying the key residues responsible for binding, make the design of β-turn mimetics quite challenging.

As may be appreciated, the present invention permits to circumvent the aforementioned difficulties by providing compounds which, thanks to their structure which incorporate numerous side chain combinations as well as multiple different side chain orientations, can be used as β-turn mimetics and evaluated accordingly.

Obviously, the preparation of libraries of β-turn mimetics represent a goal of the present invention. However, the latter is not exclusively restricted to such compounds. There are numerous other compounds according to the invention which include other interesting di- or tri-peptide motif with their structure whose active conformation need be probed by our conformation restrictive approach.

As is known many adhesive proteins present in extracellular matrices and in the blood contain the tripeptide arginine-glycine-aspartic acid (RTGD) as their cell recognition site. These proteins include fibronectin, vitronectin, osteopontin, callagens, thrombospondin, fibrinogen adn von Willebrand factor. The RGD sequence of each of the adhesive proteins are recognized by at least one member of a family of structurally related receptors, integrins, which are heterodimeric proteins with two membrane-spanning sub-units. Some of these receptors bind to the RGD sequence of a single adhesion protein only, wheras others recognize groups of them. The conformation of the RGD sequence in the individual proteins may be critical to this recognition specificity. On the cytoplasmic side of the plasma membrane the receptors connect the extracellular matrix to the cytoskeleton.

More than ten proved or suspected RGD-containing adhesion-promoting proteins have already been identified, and the integrin family includes at least as many receptors recognizing these proteins. Together the adhesion proteins and their receptors constitute a versatile recognition system providing cells with anchorage, traction for migration, and signals for polarity, position, differentiation and possibly growth. Compounds according to the invention containing the sequence Arg-Gly-Asp in a controled topology could inhibit cell to cell adhesion processes. Such compounds could be important in the areas of antithrombotic and cancer research.

Also included within the scope of the invention are compounds of the above mentioned formula (I) containing a biaryl bridge.

As can be appreciated, the compounds according to the invention have much flexibility and can adopt structures very different from conventional β-turns, according to the nature of their spacer parts. This means that the scope of the invention is broad and molecular modeling design allows the design of β and non-β-turns.

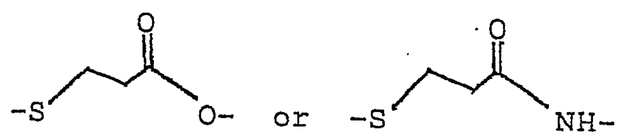
A main advantage of the invention is that the compounds of the formula (I) are neither too tight like small rings nor too loose like aliphatic chains.

The process according to the invention for preparing the above mentioned compounds basically comprises the following steps:

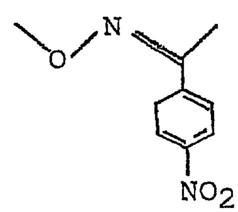
a) preparing by coupling a first building block deriving from natural or synthetic amino-acids, said first building block being of the formula:

$$\begin{array}{l} H\text{-}\underset{X}{N}\text{-}(CH_2)_x\text{-}\underset{R_1}{CH}\text{-}\underset{O}{C}\text{-}A\text{-}B\text{-}C\text{-}Sp\text{-}P \end{array}$$

wherein X, R_1, A, B, C are defined as hereinabove,

Sp is  and

P is $-CH_3$ or $-CH_2$-Ph where the coupling is carried out in liquid phase and Sp is



and P is polystyrene when the coupling is carried out in solid phase;

b) coupling the first building block prepared in step a) with a second building block hereafter called "tether", of the formula:

$$H\text{-}Y\text{-}L\text{-}Z\text{-}PG_Z$$

wherein Y, L and Z are defined as and PG_Z is a protective group; and

c) removing the protection groups PG_Z from the compound obtained in step b); and

d) carrying out a macrocyclization of the unprotected product obtained in step c) and a cleavage if the preparing and coupling steps (a), (b) were carried out in the solid phase in order to obtain the requested compound of the formula (I).

As can be noted, this process uses lactam or Mitsunob cyclization to prepare the libraries of compounds according to the invention. It is very versatile and can be carried out in a combinatorial manner, either in solid phase or in solution phase.

The invention and its advantages will be better understood upon reading the following non restrictive detailed description and examples made with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a Table detailing the structure that may have the compounds of the formula (I) according to the invention.

Figure 2a and 2b are schematic illustrations of the sequence of steps that must be carried out to obtain the library of compounds of formula (8), which is part of the "family 2" libraries shown in Figure 1 for which Y is a methylene group ($-CH_2-$), and the amino-acids at positions 1, 2 and 3 are α-amino-acids (x, y, $z = 0$).

Figure 3 is a schematic illustration of the steps that must be carried out to obtain other libraries of compounds of

formulae (9) to (12), which are also parts of the "family 2" libraries shown in Figure 1, for which Y is a methylene group ($-CH_2-$), and the amino-acids at positions 1, 2 and 3 are α-amino-acids (x, y, z = 0).

Figures 4a and 4b are schematic illustrations of the sequence of steps that must be carried out to obtain libraries of compounds of the formulae (17) and (18) which are parts of the "family 2" libraries shown in Figure 1, for which Y is a carbonyl group (-CO-), and the amino-acids at positions 1, 2 and 3 are α-amino-acids (x, y, z = 0).

DETAILED DESCRIPTION OF THE INVENTION

As aforesaid, the process according to the invention is versatile enough to prepare a large number macrocyclic compounds, the main "families" of which are illustrated in Figure 1. This process comprise the following basic steps:

a) preparing by coupling a first building block deriving from natural or synthetic amino-acids,
b) coupling the first building block prepared in step a) with a second building block called "tether",
c) removing the protective group from the compound obtained in step b), and
d) carrying out a macrocyclization of the unprotected product obtained in step c) to obtain the requested compound.

As aforesaid, the process permits to prepare a large number of compounds either in a solution phase or on a solid

support using an IRORI combinatorial chemistry set up or an other set up like an Argonault apparatus.

In the synthesis shown in Figure 2a, a first suitably protected amino-acid identified as "**A**" is activated as a thioester (solution phase or solid phase) or as an oxime ester (Kaiser resin solid phase support) to give a compound of formula (**1**). The amine protection (PGα in the case of α-amino-acids PGβ in the case of β-amino-acids and PGγ in the case of γ-amino-acids) is removed to give the compound of formula (**2**). A second amino-acid identified as "**B**" is then added in the same way to give a compound of formula (**3**), followed again by removal of the amine protection to give a compound of formula (**4**). A third acid of formula (**C**) (figure 2b) is coupled to the amine of formula (**4**) to yield a sulfonamide of the formula (**5**), which is immediately coupled with an alcohol of the formula (**D**) under Mitsunobu conditions to give a compound of formula (**6**). This compound of formula (**6**) can also be obtained directly from the compound of formula (**4**) by peptide coupling with an acid of formula (**E**). The terminal alcohol (Z = O) or amine (Z = NH) protecting group (PGz) is then cleaved to give the corresponding alcohol or amine of formula (**7**), which can undergo cyclization and cleavage all at once to give requisted compound according to the invention of formula (**8**).

As shown in Figure 3, the orthogonal protections (PG1, PG2, PG3 and PG4 when a fourth amino-acid is introduced) of the compound of formula (**8**) can be removed to yield the compound according to the invention of formula (**9**). Another

compound according to the invention of formula (**10**) can be obtained from the compound of formula (**8**) by cleaving the sulfonamide portion of the molecules. The resulting free amine can be coupled with various acids (see X groups in figure 1) to yield a compound of formula (**11**) according to the invention. Subsequent cleavage of the orthogonal protecting groups (PG0 when X is an amino-acid, PG1, PG2, PG3 and PG4 when a fourth amino-acid is introduced) yield a compound of formula (**12**) according to the invention.

As shown in figure 4, it is also possible from the amine of formula (**4**) to couple an amino-acid of formula (**C'**) to yield a compound of formula (**13**) according to the invention, whose amine protecting group (PGa, PGb or PGg) can be cleaved to give the amine of formula (**14**). A hydroxy-acid (Z = O) or amino-acid (Z = NH) of formula (**D'**) is then coupled to yield a compound according to the invention of formula (**15**). The terminal alcohol (Z = O) or amine (Z = NH) protecting group (PGz) is then cleaved to give the corresponding alcohol or amine of formula (**16**), which can undergo cyclization and cleavage all at once to yield a compound according to the invention of formula (**17**). The orthogonal protections (PG1, PG2, PG3 and PG4 when a fourth amino-acid is introduced) of the compound of formula (**17**) see figure 4b can be removed to yield a compound of formula (**18**).

The above example of synthesis deals with the preparation of libraries of the "family 2" type for which positions 1, 2 and 3 are filled. Libraries of "families 1, 3 and 4" type as shown in Figure 1 can be prepared exactly in the same way. In "family 1" type libraries, an extra amino-acid is

incorporated at position 4. In "family 3" type libraries, positions 3 and 4 are empty and in "family 4" type libraries positions 2, 3 and 4 are empty.

Thus, it is possible to develop chemical libraries of dozens, hundreds, and even many thousands of discrete chemical compounds, in an efficient and reliable manner. This being the case, it is possible to use these libraries as chemical intermediates for the preparation of pharmaceutical compounds or for the identification of such pharmaceutical compounds or other useful species. Some of these compounds could also be used without further modification. Accordingly, the ability to prepare such complex libraries in a reliable and predictable fashion is highly desired.

The term "pharmaceutical" as used herein means the ability of the compounds to provide some therapeutic or physiological beneficial effect. As used herein, the term includes any physiologically or pharmacologically activity that produces a localized or systemic effect or effects in animals including warm blooded mammals such as humans. Pharmaceutically active agents may act on the peripheral nerves, adrenegic receptors, cholinergic receptors, the skeletal muscles, the cardiovascular system, smooth muscles, the blood circulatory system, synoptic sites, neuroeffector junctional sites, endocrine and hormone systems, the immunological system, the reproductive system, the skeletal system, the autocoid system, the alimentary and excretory systems, the histamine system and central nervous systems as well as other biological systems. Thus, compounds derived from compositions of the present

invention may be used as sedatives, psychic energizers, tranquilizers, anticonvulsant, muscle relaxants, anti-Parkinson agents, analgesics, anti-inflammatories, local anesthetics, muscle contractants, antibiotic, antiviral, antiretroviral, antimalarials, diuretics, lipid regulating agents, antiandrogenic agents, antiparasities, neoplastics and chemotherapy agents. These compounds could further be used to treat cardiovascular diseases, central nervous system diseases, cancer metabolic disorders, infections and dermatological diseases as well as other biological disorders and infections.

Among the potential uses of the compounds according the present invention are uses in scientific research as research reagents. In accordance with the present invention, it is now possible to prepare pluralities of compounds to create libraries of compounds for research. Such libraries are known to be useful and are important in the discovery of new drugs. In view of the chemical and conformational diversities of such compounds e.g. the large number of functionalizable sites, a very large number of different compounds can be prepared. Moreover, such compounds can be prepared differentially, that is, in such a fashion that a population of known species can be prepared reliably, ensuring that all potential members of a family of chemical species are in fact synthesized.

In view of the foregoing, persons of ordinary skilled in the art will know how to synthesize such libraries, comprising chemical compositions within the scope and spirit of this invention and to assay the libraries against etiological agents or in tests or assays, in order to

identify compounds having antibacterial, antifungal, antiviral, antineoplastic or other desired pharmaceutical, biological, or chemical activity.

SPECIFIC EXAMPLES

Preparation of macrocyclic compounds according to the invention by the process outlined above will be illustrated by the following non-limiting specific examples :

Example 1

Library of 135 members (solution phase) (see Figure 2a)

This library (family 2 type) consists of a linear sequence of three natural L-α-amino- acids linked together by an aliphatic chain with 4 or 5 carbons in a head to tail manner. The first amino acids (**AA_1**) are glycine, leucine and methionine, the second ones (**AA_2**) are glycine, histidine(Doc), leucine, proline and valine, and the third ones (**AA_3**) are gylcine, methionine and phenylalanine.

The three third amino acids (Boc-AA3) were converted to their thioesters by coupling with methyl 3-mercapto-propionate. The formed compounds were coupled with the second five amino acids to produce 15 dipeptides which were then converted to 135 linear tripeptides by coupling with nine N-alkylated N-betsyl amino-acids. The end-to-end cyclization of the linear tripeptide thioesters was achieved by silver cation-assisted lactamization.

Amino acid, thioesters ($Boc\text{-}AA_3\text{-}S(CH_2)_2CO_2Me$):

Chlorotrimethylsilane (276 mL, 2.18 mol) was added slowly to a solution of 3-mercaptopropionic acid (70 g, 0.66 mol) in methanol (267 mL, 6.6 mol) at -10°C. The reaction mixture was then stirred for 1 h at 0°C and for 1 h at room temperature. The mixture was neutralized with saturated aqueous sodium bicarbonate to pH 8 and extracted with dichloromethane (3 X 300 mL). The combined organic phase was washed with saturated aqueous sodium bicarbonate (2 X 200 mL), brine (2 X 200 mL), dried over magnesium sulfate, filtered and evaporated under reduced pressure. The residue was then distilled (70°C/20 mmHg) to give methyl 3-mercaptopropionate as a colorless oil (61.2 g) in the yield of 77%.

1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (42 mmol) was added to a solution of N-Boc amino-acid (33 mmol), methyl 3-mercaptopropionate (30 mmol) and 4-dimethylaminopyridine (3 mmol) in dichloromethane (80 mL) at 0°C. The resulting mixture was stirred for 1 hour at 0° C and 30 min at room temperature. The reaction mixture was diluted with ethyl acetate (200 mL), washed with 1N hydrochloric acid (2 X 50 mL), saturated aqueous sodium bicarbonate (2 X 50 mL), brine (2 X 50 mL), dried over magnesium sulfate, and evaporated to give the desired thioester (**$Boc\text{-}AA_3\text{-}S(CH_2)_2CO_2Me$**) in yields ranging from 95 to 100%.

Dipeptides ($Boc\text{-}AA_2\text{-}AA_3\text{-}S(CH_2)_2CO_2Me$):

To a solution of the N-Boc amino acid thioester ($Boc\text{-}AA_3\text{-}S(CH_2)_2CO_2Me$) (5 mmol) in dichloromethane (2 mL), triethylsilane (10 mmol) was added , followed by trifluoro-acetic acid (3 mL). The reaction mixture was stirred for 1 h at room temperature, then diluted with toluene (2X10 mL) and the solvent was evaporated to give the TFA salt of $H\text{-}AA_3\text{-}S(CH_2)_2CO_2Me$.

To a solution of N-Boc amino acid ($Boc\text{-}AA_2\text{-}OH$) (5 mmol) and 1-hydroxybenzotriazole (5 mmol) in tetrahydrofuran (5 mL) and dichloromethane (5 mL) at 0°C, 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride (7 mmol) was added. The resulting mixture was stirred for 5 min at 0° C and for 20 min at room temperature, then cooled down to 0°C.

A solution of the TFA salt of $H\text{-}AA_3\text{-}S(CH_2)_2CO_2Me$ (5 mmol) in dichloromethane (5 mL) was added to the above reaction mixture at 0°C, followed by diisopropylethylamine (7.5 mmol). The resulting reaction mixture was then stirred at the same temperature for 10 min. The ice-water bath was removed and the reaction was stirred for 2 to 4 h at room temperature. Finally, the reaction mixture was diluted with ethyl acetate (80 mL) and washed with 1N hydrochloric acid (2 X 15 mL), saturated aqueous sodium bicarbonate (2 X 15 mL), brine (2 X 20 mL), dried over magnesium sulfate, and evaporated to give the dipeptide ($Boc\text{-}AA_2\text{-}AA_3\text{-}S(CH_2)_2CO_2Me$) in yields ranging from of 80 to 100%.

<u>Alkylated tripeptides (N-Bts-(N-alkylated)-AA_1-AA_2-AA_3-$S(CH_2)_2CO_2Me$):</u>

To a solution of N-Boc dipeptide (0.5 mmol) in dichloromethane (1 mL), triethylsilane (2 mmol) was added, followed by trifluoroacetic acid (1 mL). The reaction mixture was stirred for 1 h at room temperature and then diluted with toluene (2X5 mL) and evaporated to give the dipeptide TFA salt.

To a solution of alkylated N-Betsyl amino acid (0.5 mmol) and 1-hydroxybenzotriazole (0.5 mmol) in tetrahydrofuran (2 mL) and dichloromethane (2 mL) at 0°C, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.7 mmol) was added. The resulting mixture was stirred for 5 min at 0°C then for 30 min at room temperature, and cooled down to 0°C.

A solution of the dipeptide TFA salt (0.5 mmol) in dichloromethane (2 mL) was added to the above reaction mixture at 0°C, followed by diisopropylethylamine (0.8 mmol). The reaction mixture was then stirred for 10 min at the same temperature. The ice-water bath was removed and the reaction was stirred for 2 to 4 h at room temperature. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with 1N hydrochloric acid (2 X 10 mL), saturated aqueous sodium bicarbonate (2 X 10 mL), brine (2 X 10 mL), dried over magnesium sulfate, and evaporated to give the alkylated tripeptides (**Bts-(N-alkylated)-AA_1-AA_2-AA_3-$S(CH_2)_2CO_2Me$**) with yields ranging from 11 to 100%.

Preparation of cyclotripeptides :

To a solution of the alkylated tripeptide **(Bts-(N-alkylated)-AA_1-AA_2-AA_3-S$(CH_2)_2CO_2Me$)** (0.5 mmol) in dichloromethane (1 mL), triethylsilane (2 mmol) was added, followed by trifluoroacetic acid (1 mL). The reaction mixture was stirred for 1 h at room temperature and then diluted with toluene (2 X 5 mL) and the solvent was evaporated to give the TFA salt of alkylated tripeptide.

To a solution of the TFA salt of alkylated tripeptide (0.5 mmol) in ethyl acetate (250 mL), diisopropylethylamine (1.0 mmol) and silver trifluoroacetate (1.5 mmol) were added. The reaction mixture was stirred for 1 to 3 h at room temperature (Thin Layer Chromatography monitoring of the reaction). Brine (50 mL) and 1.0 M aqueous sodium thiosulfate (30 mL) was added and stirred for 60 min. The organic phase was washed with saturated EDTA aqueous solution (2 X 50 mL), 1N hydrochloric acid (50 mL), brine (2 X 50 mL), dried over magnesium sulfate and evaporated to give the crude product. The crude can be purified by flash column chromatography if necessary.

Name	MW	Quantity (mg)	Yield (%)	Purity Level (%)	Name	MW	Quantity (mg)	Yield (%)	Purity Level (%)
c-B-GGG-1	451	25	14	100	*c-B-MLG-1*	581	200	93	91
c-B-GGM-1	525	170	77	94	*c-B-MLM-1*	655	162	61	99
c-B-GGF-1	541	60	44	96	*c-B-MLF-1*	671	150	89	97
c-B-LGG-1	507	180	87	92	*c-B-GPG-1*	491	26	21	85
c-B-LGM-1	581	95	40	95	*c-B-GPM-1*	565	121	nd	87
c-B-LGF-1	597	93	83	93	*c-B-GPF-1*	581	80	62	89
c-B-MGG-1	525	80	35	96	*c-B-LPG-1*	547	73	63	97
c-B-MGM-1	599	66	28	94	*c-B-LPM-1*	621	28	31	55
c-B-MGM-1	599	31	10	77	*c-B-LPF-1*	637	25	9	85
c-B-MGF-1	615	82	27	97	*c-B-LPF-1*	637	135	64	92
c-B-MGF-1	615	25	6	69	*c-B-MPG-1*	565	102	58	91
c-B-GHG-1	673	107	53	91	*c-B-MPM-1*	639	10	98	99
c-B-GHM-1	747	97	59	98	*c-B-MPF-1*	655	140	61	76
c-B-GHF-1	763	177	68	91	*c-B-GVG-1*	493	100	56	100
c-B-LHG-1	729	82	61	85	*c-B-GVM-1*	567	13	9	59
c-B-LHM-1	803	133	65	80	*c-B-GVF-1*	583	142	75	80
c-B-LHF-1	819	162	74	97	*c-B-LVG-1*	549	142	77	96
c-B-MHG-1	747	177	63	97	*c-B-LVM-1*	623	78	95	90
c-B-MHM-1	821	160	44	90	*c-B-LVF-1*	639	290	85	89
c-B-MHF-1	837	201	51	91	*c-B-MVG-1*	567	303	95	90
c-B-GLG-1	507	229	90	97	*c-B-MVM-1*	641	26	66	96
c-B-GLM-1	581	146	68	99	*c-B-MVF-1*	657	222	64	85
c-B-GLF-1	597	130	81	80	*c-B-LLM-1*	637	123	69	95
c-B-LLG-1	563	152	88	92	*c-B-LLF-1*	653	221	97	96

Table 1. Results for the cyclic peptides with the E-alkene linker.

Name	MW	Quantity (mg)	Yield (%)	Purity (%)	Name	MW	Quantity (mg)	Yield (%)	Purity (%)
c-B-GGG-2	437	2	1	89	*c-B-MLG-2*	567	308	55	56
c-B-GGM-2	511	250	77	86	*c-B-MLM-2*	641	38	5	28
c-B-GGF-2	527	46	46	96	*c-B-MLF-2*	657	206	32	44
c-B-LGG-2	493	142	76	99	*c-B-GPG-2*	477	31	29	92
c-B-LGM-2	567	112	23	55	*c-B-GPM-2*	551	76	nd	82
c-B-LGF-2	583	64	35	67	*c-B-GPF-2*	567	55	42	92
c-B-MGG-2	511	56	49	96	*c-B-LPG-2*	533	0	nd	
c-B-MGM-2	585	28	10	87	*c-B-LPM-2*	607	34	54	49
c-B-MGM-2	585	40	14	77	*c-B-LPF-2*	623	19	8	96
c-B-MGF-2	601	75	20	94	*c-B-MPG-2*	551	46	22	78
c-B-MGF-2	601	32	9	88	*c-B-MPM-2*	625	5	1	59
c-B-GHG-2	659	80	49	93	*c-B-MPF-2*	641	44	11	49
c-B-GHM-2	733	52	22	63	*c-B-GVG-2*	479	61	23	85
c-B-GHF-2	749	159	39	66	*c-B-GVM-2*	553	59	80	93
c-B-LHG-2	715	72	47	94	*c-B-GVF-2*	569	191	86	76
c-B-LHM-2	789	111	31	51	*c-B-LVG-2*	535	92	36	89
c-B-LHF-2	805	169	46	67	*c-B-LVM-2*	609	60	57	93
c-B-MHG-2	733	30	9	98	*c-B-LVF-2*	625	152	36	87
c-B-MHM-2	807	90	6	42	*c-B-MVG-2*	553	112	30	82
c-B-MHF-2	823	159	30	69	*c-B-MVM-2*	627	6	7	48
c-B-GLG-2	493	166	97	100	*c-B-MVF-2*	643	0	nd	
c-B-GLM-2	567	143	85	97	*c-B-LLM-2*	623	85	26	61
c-B-GLF-2	583	90	32	57	*c-B-LLF-2*	639	193	43	77
c-B-LLG-2	549	110	48	94					

Table 2. Results for the cyclic peptides with the Z-alkene linker.

Name	MW	Quantity (mg)	Yield (%)	Purity (%)	Name	MW	Quantity (mg)	Yield (%)	Purity (%)
c-B-GGG-3	435	11	6	96	*c-B-LLG-3*	547	124	46	83
c-B-GGM-3	509	258	58	85	*c-B-LLG-3*	547	61	11	41
c-B-GGF-3	525	15	9	93	*c-B-LLM-3*	621	148	62	81
c-B-LGG-3	491	150	69	91	*c-B-LLF-3*	637	196	55	82
c-B-LGM-3	565	60	22	98	*c-B-MLG-3*	565	167	24	40
c-B-LGM-3	565	86	27	84	*c-B-MLM-3*	639	27	9	76
c-B-LGF-3	581	36	34	91	*c-B-MLF-3*	655	100	22	65
c-B-LGF-3	581	10	8	91	*c-B-GPG-3*	475	37	46	99
c-B-LGF-3	581	22	18	83	*c-B-GPM-3*	549	15	16	50
c-B-MGG-3	509	60	38	89	*c-B-GPF-3*	565	45	83	99
c-B-MGM-3	583	32	12	96	*c-B-LPG-3*	531	0	1	58
c-B-MGM-3	583	54	17	83	*c-B-LPM-3*	605	54	29	38
c-B-MGF-3	599	96	31	98	*c-B-LPF-3*	621	26	10	91
c-B-MGF-3	599	55	13	73	*c-B-MPG-3*	549	23	15	98
c-B-GHG-3	657	104	54	94	*c-B-MPM-3*	623	10	41	41
c-B-GHM-3	731	52	26	61	*c-B-MPF-3*	639	5	nd	
c-B-GHF-3	747	80	24	81	*c-B-GVG-3*	477	39	19	79
c-B-LHG-3	713	119	70	85	*c-B-GVM-3*	551	33	34	66
c-B-LHM-3	787	173	66	73	*c-B-GVF-3*	567	36	6	28
c-B-LHF-3	803	224	64	75	*c-B-LVG-3*	533	158	64	82
c-B-MHG-3	731	107	29	93	*c-B-LVM-3*	607	75	64	83
c-B-MHM-3	805	97	13	78	*c-B-LVF-3*	623	356	64	79
c-B-MHF-3	821	163	38	83	*c-B-MVG-3*	551	285	63	62
c-B-GLG-3	491	63	38	100	*c-B-MVM-3*	625	8	9	50
c-B-GLM-3	565	53	15	51	*c-B-MVF-3*	641	123	31	77
c-B-GLF-3	581	20	7	44					

Table 3. Results for the cyclic peptides with the alkyne linker.

Spectral Data for betsylated c-Met-Leu-Phe-Linker compounds:

Trans-linker (c-B-MLF-1) :

^1H NMR ($CDCl_3$, 300 MHz): 8.32 (1 H, br), 8.19 (1 H, dd, J = 8.0 and 1.7 Hz), 8.01 (1 H, dd, J = 8.5 and 1.7 Hz), 7.70-7.59 (2 H, m), 7.34-7.22 (5 H, m), 6.91 (1 H, br), 6.66 (1H, br), 5.70-5.60 (1 H, m), 5.21-5.16 (1 H, m), 4.64-4.59 (2 H, m), 3.92-3.85 (3 H, m), 3.35-3.20 (2 H, m), 3.30 (1 H, dd, J = 14.0 and 4.7 Hz), 3.16 (1 H, dd, J = 13.9 and 9.3 Hz), 2.47-2.32 (3 H, m), 2.09-2.04 (3 H, m), 1.97 (3 H, s), 1.73-1.44 (3 H, m), 0.86 (3 H, d, J = 6.3 Hz), 0.81 (3 H, d, J = 6.3 Hz).

LC-MS : m/e : 671 (M^+)

Cis-linker (c-B-MLF-2) :

^1H NMR ($CDCl_3$, 300 MHz): 8.65 (1 H, J = 5.9 Hz), 8.05 (1 H, dd, J = 8.9 and 1.6 Hz), 8.00 (1 H, d, J = 8.1 Hz), 7.66-7.57 (2 H, m), 7.33-7.22 (3 H, m), 7.16 (2 H, d, J = 7.1 Hz), 6.71-6.68 (2 H, m), 5.72-5.56 (2 H, m), 5.01 (1 H, t, J = 7.3 Hz), 4.54 (1 H, dt, J = 8.7 and 4.9 Hz), 4.08-4.00 (2 H, m), 3.94 (1 H, dd, J = 16.4 and 7.3 Hz), 3.67-3.56 (2 H, m), 3.23 (1 H, dd, J = 14.1 and 4.8 Hz), 3.00 (1 H, dd, J = 14.1 and 9.0 Hz), 2.67-2.47 (2 H, m), 2.36-2.11 (1 H, m), 2.09 (3 H, s), 2.07-1.99 (1 H, m), 1.92-1.82 (1 H, m), 1.61-1.52 (1 H, m), 1.49-1.41 (1 H, m), 0.93 (3 H, d, J = 6.5 Hz), 0.87 (3 H, d, J = 6.3 Hz).

LC-MS : m/e : 657 (M^+).

Acetylene-linker (c-B-MLF-3) :

^1H NMR ($CDCl_3$, 300 MHz): 8.32(2 H, d, J = 8.1 Hz), 8.01 (1 H, d, J = 8.0 Hz), 7.72-7.60 (2 H, m), 7.49 (1 H, br), 7.31-7.20 (5 H, m), 6.80 (1 H, br), 4.80-4.76 (1 H, m),

4.42-4.36 (2 H, m), 4.13-3.95 (3 H, m), 3.45-3.40 (1 H, m), 3.29 (1 H, dd, J = 14.2 and 5.6 Hz), 3.14 (1 H, dd, J = 14.1 and 10.1 Hz), 2.54-2.47 (3 H, m), 2.12-2.08 (1 H, m), 1.97 (3 H, s), 1.74 (2 H, t, J =7.6 Hz), 1.42-1.33 (1 H, m), 0.82 (3 H, d, J = 6.6 Hz), 0.78 (3 H, d, J = 6.6 Hz). LC-MS : *m/e* : 655 (M^+).

Example 2

Removal of betsyl group of cyclotripeptides

1) in solution :

Potassium trimethylsilanolate (0.2 mmol) was added to a solution of 2-naphthalenethiol (0.2 mmol) in a deoxygenated mixture of THF and EtOH (1:1, 1 mL) at room temperature and the resulting mixture was stirred for 20 min. N-Bts-cyclotripeptide (0.1 mmol) was then added. The resulting mixture was stirred for 1 h and evaporated to dryness. The residue was purified by column to give the deprotected product with yields ranging from 77 to 86%.

2) on solid support :

Polystyrene-thiophenol Resin (0.2 mmol) was added to a solution of potassium trimethylsilanolate (0.2 mmol) in a deoxygenated mixture of THF and EtOH (1:1, 1 mL) at room temperature and the resulting mixture stirred for 20 min. The solution was removed by filtration. The resin was washed with a deoxygenated mixture of THF and EtOH (1:1, 3 X 2 mL) and added to a solution of N-Bts-cyclotripeptide (0.1 mmol) in a deoxygenated mixture of THF and EtOH (1:1, 1 mL). The resulting mixture was stirred for 1 h. The resin was removed by filtration and washed with THF and

EtOH (1:1 mixture, 2 X 2 mL). The filtrate was concentrated to give the crude product in quantitive yield.

Data for c-Met-Leu-Phe-Linker compounds:

Trans-linker (c-MLF-1) :

^{1}H NMR ($CDCl_3$, 300 MHz): 7.77 (1 H, d, J = 7.7 Hz), 7.30-7.14 (5 H, m), 6.65 (1 H, d, J = 7.4 Hz), 6.53 (1H, br), 5.56-5.64 (1 H, m), 5.32-5.22 (1 H, m), 4.03-3.95 (2 H, m), 3.79-3.68 (1 H, m), 3.57-3.35 (3 H, m), 3.12-3.02 (2 H, m), 2.96-2.86 (1 H, m), 2.61-2.50 (2 H, m), 2.24-1.98 (3 H, m), 2.10 (3 H, s), 1.78 (2 H, m), 1.55-1.39 (2 H, m), 0.86 (3 H, d, J = 6.6 Hz), 0.83 (3 H, d, J = 6.8 Hz).

LC-MS : *m/e* : 474 (M^+)

Cis-linker (c-MLF-2) :

^{1}H NMR ($CDCl_3$ & CD_3OD, 300 MHz): 7.27-7.14 (5 H, m), 5.80-5.71 (1 H, m), 5.68-5.53 (1 H, m), 4.21-4.09 (2 H, m), 3.88 (1 H, dd, J = 14.0 and 6.0 Hz), 3.64 (1 H, dd, J = 14.0 and 7.3 Hz), 3.34-3.15 (5 H, m), 2.52 (2 H, t, J = 7.3 Hz), 2.07 (3 H, s), 2.00-1.86 (2 H, m), 1.53-1.15 (3 H, m), 0.80 (6H, d, J = 6.6 Hz).

LC-MS : *m/e* : 460 (M^+).

Acetylene-linker (c-MLF-3) :

^{1}H NMR ($CDCl_3$, 300 MHz): 7.92 (1 H, d, J = 8.8 Hz), 7.34 (1 H, d, J = 9.2 Hz), 7.31-7.17 (5 H, m), 7.02 (1 H, t, J = 5.9 Hz), 4.21 (1 H, q, J = 8.0 Hz),, 4.10-3.99 (2 H, m), 3.79 (1 H, dm, J = 15.5 Hz), 3.67 (1 H, dm, J = 16.0 Hz), 3.49-3.35 (3 H, m), 3.25 (1 H, dd, J = 8.8 and 4.3 Hz), 2.60-2.54 (2 H, m), 2.15-1.99 (1 H, m), 2.11 (3 H, s),

1.80-1.68 (2 H, m), 1.57-1.46 (2 H, m), 0.86 (6 H, d, *J* = 6.3 Hz).
LC-MS : *m/e* : 458 (M^+).

Example 3

Preparation of N-Formyl-cyclotripeptides

The free amine macrocycle (see example 2) was added to a mixture of formic acid (0.2 mL) and acetic anhydride (0.1 mL) and the reaction mixture was stirred for 15 min at room temperature, then for 5 min at 55° C and finally for 2h at room temperature. The reaction mixture was evaporated to dryness and purified by column chromatography to give the desired compounds in yields ranging from 81 to 100%

Data for formylated c-Met-Leu-Phe-Linker compounds:

Trans-linker (c-f-MLF-1) :
^{1}H NMR ($CDCl_3$, 300 MHz): 8.72 (1 H, s), 7.92 (1 H, s), 7.38-7.22 (5 H, m), 6.78 (1 H, br), 5.83 (1 H, d, *J* =10.0 Hz), 5.68-5.58 (1 H, m), 4.96-4.89 (1 H, m), 4.02-3.68 (5 H, m), 3.34 (1 H, dd, *J* = 14.0 and 10.0 Hz), 2.78 (1 H, dd, *J* = 13.5 and 5.2 Hz), 2.67-2.51 (3 H, m), 2.42-2.31 (1 H, m), 2.18-2.04 (1 H, m), 2.06 (3 H, s), 1.99-1.53 (6 H, m), 1.03 (3 H, d, *J* = 6.0 Hz), 0.94 (3 H, d, *J* = 6.0 Hz).
LC-MS : *m/e* : 502 (M^+) (84.2 %).

Cis-linker (c-f-MLF-2) :
^{1}H NMR ($CDCl_3$, 300 MHz): 8.04 (1 H, s), 7.29-7.18 (5 H, m), 6.82-6.74 (1 H, m), 6.39 (1 H, d, *J* = 9.5 Hz), 6.16 (1 H, dd, *J* = 18.0 and 7.5 Hz), 6.94-6.85 (1 H, m), 4.80-4.72 (1 H, m), 4.24-3.68 (5 H, m), 3.50-3.43 (1 H, m), 2.87-2.79 (1

H, m), 2.61-2.45 (3 H, m), 2.14-2.01 (1 H, m), 2.11 (3 H, s), 1.74-1.62 (1 H, m), 1.43-1.25 (3 H, m), 0.93-0.78 (6 H, m).
LC-MS : *m/e* : 488 (M^+).

Acetylene-linker (c-f-MLF-3) :
^{1}H NMR ($CDCl_3$, 300 MHz): 8.10 (1 H, s), 7.83 (1 H, br), 7.31-7.16 (5 H, m), 6.97 (1 H, br), 6.45 (1 H, br), 4.35-4.01 (5 H, m), 3.87-3.71 (2 H, m), 3.38 (1 H, dd, J = 14.5 and 5.0 Hz), 3.24-3.16 (1 H, m), 2.59-2.23 (3 H, m), 2.08 (3 H, s), 1.66-1.25 (4 H, m), 0.84 (6 H, t, J = 6.0 Hz).
LC-MS : *m/e* : 486 (M^+).

Example 4

Solid Phase Synthesis on the Kaiser Resin

Anchoring Boc-amino acid to the resin :

To the Kaiser resin (2.0g, 0.95 mmol/g) was added a 0.2M solution of N-Boc amino acid and 4-dimethylaminopyridine (0.25eq). After shaking for 5 min, diisopropylcarbodiimide (1.5 eq) was added and the reaction mixture was agitated for 16 h. The resin was washed with dichloromethane (3 X 30 mL), tetrahydrofuran (1 X 30 mL), methanol (1 X 30 mL), dichloromethane (1 X 30 mL), methanol (1 X 20 mL), tetrahydrofuran (1 X 30 mL), methanol (1 X 20 mL), dichloromethane (2 X 30 mL) and dried by nitrogen flow. The unreacted hydroxy group on the resin was then capped by reacting with acetic anhydride (5 mL) and diisopropylethylamine (1 mL) in dichloromethane (20 mL) at room temperature for 2 h. The resin was washed and dried by the same procedure mentioned above. The substitution level was 0.2-0.3 mmol/g.

Formation of dipeptides (Performed on the Quest 210™):

25 % TFA in dichloromethane (20 mL) was added to the above resin (0.4 mmol, 2.0 g, 0.2 mmol/g) and agitated for 30 min. The resin was then washed with dichloromethane (3 X 30 mL), methanol (1 X 20 mL), dichloromethane (1 X 30 mL), methanol (1 X 20 mL), dichloromethane (1 X 30 mL), methanol (1 X 20 mL), dichloromethane (2 X 30 mL) and dried by nitrogen flow.

A 0.2M solution of hydroxybenzotriazole and diisopropyl-carbodiimide in 60% dichloromethane/tetrahydrofuran was added to the N-Boc amino acid, followed by diisopropyl-ethylamine (1.5eq). The resulting mixture was stirred for 30 min at room temperature, and then transfered to the resin (200 mg on Quest 210™) and agitated at room temperature for 30 min. Diisopropylethylamine (2.0 mmol) was then added and agitated until Kaiser test of an aliquot of the resin was negative(2 to 4 h). The resin was washed with dichloromethane (3 X 4 mL), tetrahydrofuran (1 X 4 mL), methanol (1 X 4 mL), dichloromethane (1 X 4 mL), methanol (1 X 4 mL), tetrahydrofuran (1 X 4 mL), methanol (1 X 4 mL), dichloromethane (2 X 4 mL) and dried by nitrogen flow.

Formation of tripeptides :

25 % TFA in dichloromethane (4 mL) was added to the Boc-protected dipeptide resin (0.04 mmol, 200mg g, 0.2 mmol/g) and agitated for 30 min. The resin was then washed with dichloromethane (3 X 4 mL), methanol (1 X 4 mL), dichloromethane (1 X 4 mL), methanol (1 X 4 mL), dichloromethane (1 X 4 mL), methanol (1 X 4 mL), dichloromethane (2 X 4 mL) and dried by nitrogen flow.

A 0.2M solution of hydroxybenzotriazole and diisopropylcarbodiimide in 60% dichloromethane/tetrahydrofuran was added to the N-Bts amino acid, followed by diisopropylethylamine (1.5eq). The resulting mixture was stirred for 30 min at room temperature, and then transferred to the resin (200 mg on Quest 210™) and agitated at room temperature for 30 min. The resin was washed with dichloromethane (3 X 4 mL), tetrahydrofuran (1 X 4 mL), methanol (1 X 4 mL), dichloromethane (1 X 4 mL), methanol (1 X 4 mL), tetrahydrofuran (1 X 4 mL), methanol (1 X 4 mL), dichloromethane (2 X 4 mL) and dried by nitrogen flow.

Mitsunobu reaction :

A 0.2M solution of 5-(*tert*-butoxycarbonylamino)-*trans*-2-penten-1-ol or N-*tert*-butoxycarbonyl-2-(2-hydroxyethoxy)-cinnamyl amine, and triphenylphosphine was added to the tripeptide resin (0.036 mmol, 180 mg, 0.2 mmol/g on the Quest 210™) in anhydrous tetrahydrofuran, followed by diethyl azodicarboxylated (1.5eq). The mixture was agitated for 2 h and the resin was washed and dried by the same procedure mentioned above.

Cyclization of alkylated tripeptides :

The N-alkylated linear tripeptide (0.016 mmol, 80 mg) was treated with 25% TFA in dichloromethane (4 mL) for 30 min and washed with dichloromethane, methanol and dried by nitrogen flow.

Toluene(2 mL), acetic anhydride (1 mL) and diisopropylethylamine (1 mL) were added to the above resin and agitated for 2 h. The resin was removed by filtration and washed with dichloromethane (3 X 4 mL). An aliquot of the

the filtrate was analysized by LC-MS. The filtrate was concentrated and then diluted with ethyl acetate (15 mL). The solution was washed with 1N hydrochloric acid (2 mL), saturated sodium bicarbonate (2 mL), brine (3 mL), dried and evaporated to give the crude product.

SAMPLE ID	ISOLATED QUANTITY (MG)	PURITY
c-B-MPG-1	5.9 (42%)	Low
c-B-MVG-1	4.4 (48%)	Medium
c-B-MGM-1	4.6 (32%)	Low
c-B-LPM-1	7.5 (50%)	Low
c-B-LLM-1	7.1 (46%)	Good
c-B-GVM-1	3.1 (22%)	Low
c-B-GLF-1	7.8 (81%)	Good
c-B-MVG-4*	2.4 (23%)	Good
c-B-MGM-4*	5.7 (34%)	Low
c-B-LPM-4*	3.3 (19%)	Low
c-B-LMM-4*	5.0 (29%)	Medium
c-B-GVM-4*	4.0 (25%)	Low
c-B-GLF-4*	2.9 (26%)	Low

*Nature of linker 4 is shown in Scheme 2.

Table 4. Library of Macrocyclic Tripeptides Synthesized on the Kaiser Resin Using the Quest 210™:

Example 5

Synthesis of Betsylated Macrocyclic Tripeptides on Thio-ester Resin in IRORI MACROKANS™:

Macrocyclic tripeptides were synthesized in MACROKANS™ (160 mg of aminomethyl resin) following the same procedure as that given for the synthesis on solid support (KAISER Resin, see example 4) :

SAMPLE ID	Quantity (mg)	PURITY
c-B-GHG-1	7.6 (6%)	Good
c-B-GLF-1	16.3 (14%)	Excellent
c-B-GVM-1	20 (18%)	Good
c-B-LHF-1	43 (27%)	Excellent
c-B-LPM-1	26.7 (22%)	Excellent
c-B-LLM-1	20.6 (17%)	Excellent
c-B-MVG-1	10.5 (10%)	Good
c-B-MPG-1	6.4 (6%)	Low
c-B-GHG-4	12.4 (11%)	Excellent
c-B-GLF-4	12.4 (9%)	Excellent
c-B-GVM-4	9.3 (7%)	Good
c-B-LHF-4	21.8 (16%)	Excellent
c-B-LPM-4	29.1 (21%)	Excellent
c-B-LLM-4	25.8 (18%)	Excellent
c-B-MVG-4	37.5 (30%)	Excellent
c-B-MPG-4	30.3 (24%)	Excellent

Table 5. Library of Macrocyclic Tripeptides Synthesized

Example 6

Synthesis of Mesylated Macrocyclic Tripeptides on Thioester Resin in IRORI MINIKANS™

Macrocyclic tripeptides were synthesized in MINIKANS™ (60mg of aminomethyl resin) following the same procedure as that given for the synthesison solid support (KAISER Resin, see example 4) :

SAMPLE ID	Quantity (mg)	PURITY
c-Ms-GLG-4	3 (9%)	Low
c-Ms-GLG-1	3 (12%)	Low
c-Ms-E(OMe)LG-4	1.9 (5%)	Good
c-Ms-E(Ome)LG-1	4 (13%)	Good
c-Ms-LPM-4	0.1	Good
c-Ms-LPM-1	0.5	Low
c-Ms-YLG-4	2 (5%)	Good
c-Ms-YLG-1	6 (18%)	Good
c-Ms-GLF-4	6 (16%)	Good
c-Ms-GLF-1	5 (15%)	Good
c-Ms-LLG-4	1 (3%)	Low
c-Ms-LLG-1	--	Low
c-Ms-LLM-4	--	Low
c-Ms-SLG-4	--	Good
c-Ms-E(COOH)LG-4	13.3 *	Good
c-Ms-E(COOH)LG-1	21*	Good

*Unpurified

Table 6. Library of Macrocyclic Tripeptides Synthesized

SPECIFIC EXAMPLES OF BUILDING UNITS

Tether building blocks (type **D**, see figure 2b)

The tether building blocks have the following formula: HO-Y-L-Z-PGz.

The three following tether building blocks 4-(*tert*-butoxycarbonylamino)-*cis*-2-buten-1-ol ($Y=CH_2$, L=[Z]alkene, $Z=CH_2$) and 4-(*tert*-butoxycarbonylamino)-2-butyn-1-ol ($Y=CH_2$, L=alkyne, $Z=CH_2$) and 5-(*tert*-butoxycarbonylamino)-*trans*-2-penten-1-ol ($Y=CH_2$, $L=CH_2$-[E]alkene, $Z=CH_2$) were synthesized from commercial available *cis*-2-butene-1,4-diol, 2-butyne-1,4-diol and 3-amino-1-propanol respectively.

Preparation of 4-(*tert*-butoxycarbonylamino)-2-butyn-1-ol : Dowex 50WX8-100 ion-exchange resin (53 g) was added to a suspension of 2-butyne-1,4-diol (153.9 g, 1.77 mol) and dihydropyran (250 mL, 2.66 mol) in dichloromethane (800 mL) at room temperature. The resulting mixture was stirred for 60 min and quenched with triethylamine (10 mL). The resin was removed by filtration. The filtrate was washed with a saturated aqueous solution of sodium bicarbonate (100 mL), brine (3 X 300 mL), dried over magnesium sulfate, filtered and evaporated under reduced pressure to give the desired monoprotected 2-butyne-1,4-diol with a yield of 50%.

To a solution of monoprotected 2-butyne-1,4-diol (20.4 g, 0.12 mol) and triphenylphosphine (40.9 g, 0.16 mol) in tetrahydrofuran (50 mL), hydrazoic acid (113 mL, 1.6 M in toluene, 0.18mol) was added at 0°C. Diisopropyl azodi-

carboxylate (29.5 mL, 0.15 mol) was added dropwise to the solution whose temperature was around 0°C. The reaction was stirred for 30 min at 0°C and for 30 min at room temperature. Triphenylphosphine (40.9 g, 0.16 mol) was added at 0°C and the reaction was stirred overnight at room temperature. After addition of water (50 mL), the mixture was heated at 60°C for 4 h, 1N hydrochloric acid (140 mL) was added. After stirring for 1 h, brine (140 mL) and dichloromethane (500 mL) were added. The aqueous phase was washed with dichloromethane (3 X 100 mL). Potassium carbonate (16.5 g, 0.12 mol) was added, followed by a solution of di-*tert*-butyl dicarbonate (26.2, 0.12 mol) in tetrahydrofuran (100 mL). The reaction mixture was stirred for 18 h, then extracted with dichloromethane (1 X 300 mL, 2 X 50 mL). The combined organic extract was washed with brine (50 mL), dried over magnesium sulfate, filtered and evaporated under reduced pressure. The residue was purified by a dry-pack silica gel column to give the titled compound in 50% yield.

Preparation of 4-(*tert*-butoxycarbonylamino)-*cis*-2-buten-1-ol :

The title compound was synthesized from *cis*-2-butene-1,4-diol in an overall yield of 30% according to the procedure used for 4-*tert*-butoxycarbonylamino-2-butyn-1-ol .

Preparation of 5-(*tert*-butoxycarbonylamino)-*trans*-2-penten-1-ol:

A solution of di-*tert*-butyl dicarbonate (382.8 g, 1.75 mol) in dichloromethane (1.6 L) was added to 3-amino-1-propanol (263.6 g, 3.51 mol) during 2 h. The reaction mixture was stirred for an additional 40 min and water (1 L) was added.

The organic phase was washed with water (3 X 500 mL),), dried over magnesium sulfate, filtered and evaporated under reduced pressure to give 3-(*tert*-butoxycarbonylamino)-1-propanol in 96% yield.

Dimethyl sulfoxide (321 mL, 4.5 mol) and triethylamine (941 mL, 6.75 mol) were added to a solution of 3-*tert*-butoxycarbonylamino-1-propanol (262.6 g, 1.5 mol) in dichloromethane (1.2 L) at 0°C. Sulfur trioxide pyridine complex (286.4 g, 1.8 mol) was added in 6 portions. The reaction mixture was then stirred for 30 min at 0°C and for 30 min at room temperature. The reaction was cooled down to 0°C, quenched with 1N hydrochloric acid, washed with brine (2 X 500 mL), dried over magnesium sulfate, filtered and evaporated under reduced pressure to give 3-(*tert*-butoxycarbonylamino)propionaldehyde.

To a solution of the above crude aldehyde (1.5 mol) in acetonitrile (1.3 L), trimethyl phosphonoacetate (409.7 g, 2.25 mol) and lithium hydroxide (53.9 g, 2.25 mol) were added and stirred overnight at room temperature. The reaction was quenched with water (50 mL). The acetonitrile solvent was removed by evaporation under reduced pressure. The residue was then diluted with diethyl ether (800 mL), washed with 1N sodium hydroxide (3 X 300 mL), brine (2 X 500 mL), dried over magnesium sulfate, filtered and evaporated under reduced pressure to give the desired *trans*-unsaturated methyl ester.

Diisobutylaluminium hydride (1.12 L, 1.0 M in dichloromethane, 1.12 mol) was added dropwise to a solution of this methyl ester (102.75 g, 0.445 mol) in dichloromethane

(250 mL) at 0° C. The resulting mixture was stirred for an additional 1 h and then poured slowly into a 1M tartaric acid aqueous solution (1.4 L), extracted with dichloromethane (3 X 500 mL). The combined organic phase was dried over magnesium sulfate, filtered and evaporated under reduced pressure. The residue was purified by dry-pack silica gel column to give the titled compound with a yield of 40% for the three steps.

Amino-acid building block (type **A** and **B**, see figure 2a)

The N-Boc amino acids are commercial available except N^{α}-Boc-N^{im}-Doc-histidine which was prepared by the method described below.

Preparation of N^{α}-Boc-N^{im}-Doc-histidine :

A solution of 2,4-dimethyl-3-pentanol (83.2 g, 0.72 mol) and triethylamine (125 mL, 0.90 mol) in toluene (300 mL) was added slowly (30 min) to a solution of phosgene (531 mL, 20% in toluene, 1.07 mol) in toluene (531 mL) at 0°C. The mixture was stirred for an additional 30 min at the same temperature. Ice-cold water (500 mL) was added and the organic phase was washed with ice-cold water (2 X 100 mL), dried over magnesium sulfate, filtered and evaporated under reduced pressure. The residue was then distilled under reduced pressure (6 cm of Hg, 33-35°C) to give Doc-Cl as a colorless oil (92 g, 72%).

A solution of Doc-Cl (57.9 g, 0.32 mol) in tetrahydrofuran (250 mL) was added slowly to a solution of N^{α}-Boc-histidine (69 g, 0.27 mol) and potassium carbonate (41.1 g, 0.28 mol)

in water (350 mL) at 0°C. The resulting mixture (pH=10) was stirred for 2 h at the same temperature and for 1 h at room temperature. Water (150 mL) and hexane (200 mL) were added to the reaction mixture. The separated aqueous phase was washed with a mixture of diethyl ether and hexane (1:1; 3 X 200 mL), acidified with 20% citric acid aqueous solution to pH 2-3, and then extracted with dichloromethane (3 X 200 mL). The combined dichloromethane solution was washed with brine (200 mL), dried over magnesium sulfate, filtered and evaporated under reduced pressure to give the crude N^{α}-Boc-N^{im}-Doc-histidine in quantitive yield.

Building blocks of type C (see figure 2b)

N-Betsyl protected amino acids (Bts-AA1) were synthesized by the reaction of amino acids with Betsyl chloride (benzothiazole-2-sulfonylchloride) which was obtained from mercaptobenzothiazole and chlorine.

Preparation of N-Betsyl amino acid (Bts-AA_1OH) :

Chloride gas was bubbled into a solution of acetic acid (250mL) in water (500 mL) at 5°C until an orange precipitate was formed in good quantity. A solution of mercaptobenzothiazole (0.7 mol) in aqueous acetic acid (750 mL, 33% in water) was added in portions to the above reaction mixture in a period of 3 hours. The reaction mixture was stirred for 1 h, filtered at 0°C then washed with cold water. The solid was dissolved in cold dichloromethane (500 mL) and washed with cold brine (2 X 100 mL), cold saturated sodium bicarbonate (100 mL), brine (100 mL), dried over magnesium sulfate, filtered and

evaporated at 10°C under reduced pressure. The solid was then washed with cold diethyl ether (100 mL), cold acetonitrile (100 mL), filtered and pumped to give betsyl chloride (benzothiazole-2-sulfonylchloride).

To a solution of amino acid (0.11 mol) in 0.25 N aqueous sodium hydroxide (0.08 mol) at room temperature (initial pH around 9.5), betsyl chloride (0.1 mol) was added. The resulting mixture was stirred vigorously for 18 h. The pH of the reaction was adjusted between 9.5 to 10.0 with 1.0 N aqueous sodium hydroxide during the reaction progress. The reaction mixture was washed with diethyl ether (3 X 50 mL). The aqueous phase was then cooled to 0°C, acidified to pH 1.5-2.0 with 6 N HCl, and extracted with ethyl acetate (3 X 100 mL). The combined ethyl acetate solution was dried over magnesium sulfate, filtered and evaporated under reduced pressure to give the desired compound in 74-85% yield.

Building blocks of type E (see figure 2b)

These building blocks correspond to building blocks of type **C** which have been alkylated using Mitsunobu reaction conditions with the building blocks of type **D**. To be able to carry out this alkylation, the acid functional group of **C** must be protected. The protecting group used is finally removed to get the desired compounds

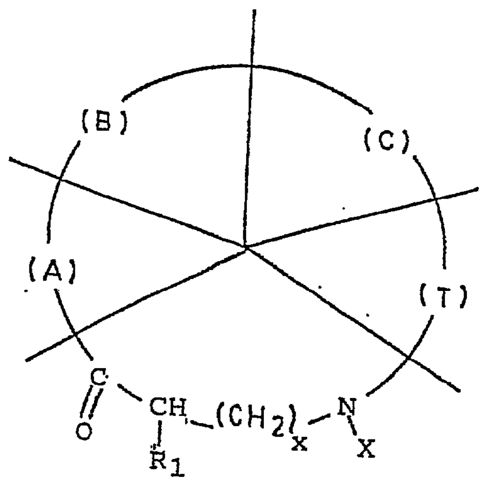
<u>Preparation of N-alkylated N-Betsyl amino acid :</u>

Dihydrofuran (90 mmol) and pyridinium p-toluenesulfonate (1.5 mmol) were added to a suspension of N-Betsyl amino acid (30 mmol) in dichloromethane (50 mL) at 0°C. The resulting mixture was stirred for 15 min at 0°C and for 60

min at room temperature. The reaction mixture was diluted with diethyl ether (150 mL), washed with saturated aqueous sodium bicarbonate (20 mL), brine (20 mL), dried over magnesium sulfate, filtered and evaporated under reduced pressure to give the tetrahydrofuranyl ester of amino acid. A mixture of this ester (30 mmol), an alcohol (type D building block) (i.e. 4-(*tert*-butoxycarbonylamino)-*cis*-2-buten-1-ol, or 4-(*tert*-butoxycarbonylamino)-2-butyn-1-ol, or 5-(*tert*-butoxycarbonylamino)-*trans*-2-penten-1-ol)) (43.5 mmol) and triphenylphosphine (49 mmol) were suspended in toluene (20 mL) and azeotropically distilled three times in vacuum. The residue was dissolved in tetrahydrofuran (40 mL). Diisopropyl azodicarboxylate (43.5 mmol) was added at 0°C. After stirring for 15 min at 0°C and for 30 min at room temperature, 1N hydrochloric acid (30 mL) and methanol (30 mL) was added and stirred for an additional 60 min. After removal of the organic solvents by evaporation, the aqueous phase was diluted with water (30 mL), the pH of the medium was adjusted to 12 with potassium carbonate, washed with diethyl ether (3 X 60 mL), and then acidified to pH 2-3 with 1N hydrochloric acid, extracted with dichloromethane (3 X 200 mL). The combined dichloromethane solution was washed with brine (100 mL), dried over magnesium sulfate, filtered and evaporated under reduced pressure to give the desired alkylated N-Betsyl amino acid in the overall yield of 68-89%.

WHAT IS CLAIMED IS:

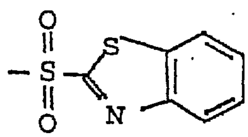
1. A macrocyclic compound of the formula (I)



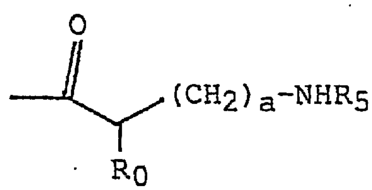
(I)

- where part (A) is a $-\underset{O}{C}-\underset{R_2}{CH}-(CH_2)_y-NH-$ bivalent radical having its -NH- group linked to the carbonyl group of part $-\underset{O}{C}-\underset{R_1}{CH}-(CH_2)_x-\underset{X}{N}-$, a $-(CH_2)_y-$ bivalent radical, or a covalent bond;
- where part (B) is a $-\underset{O}{C}-\underset{R_3}{CH}-(CH_2)_z-NH-$ bivalent radical having its -NH- group linked to part (A), a $-(CH_2)_z-$ bivalent radical, or a covalent bond;
- where part (C) is a $-\underset{O}{C}-\underset{R_4}{CH}-(CH_2)_t-NH-$ bivalent radical having its -NH- group linked to part (B), a $-(CH2)_t-$ bivalent radical, or a covalent bond;

- where part (T) is a - Y - L - Z - radical; and
- where X is a monovalent group selected from the group consisting of: $-SO_2-Ar$, $-SO_2-CH_3$, $-SO_2-CF_3$, -H, -COH, $-CO-CH_3$, -CO-Ar, -CO-R, -CO-NHR, -CO-NHAr, -CO-O-tBu, $-CO-O-CH_2-Ar$

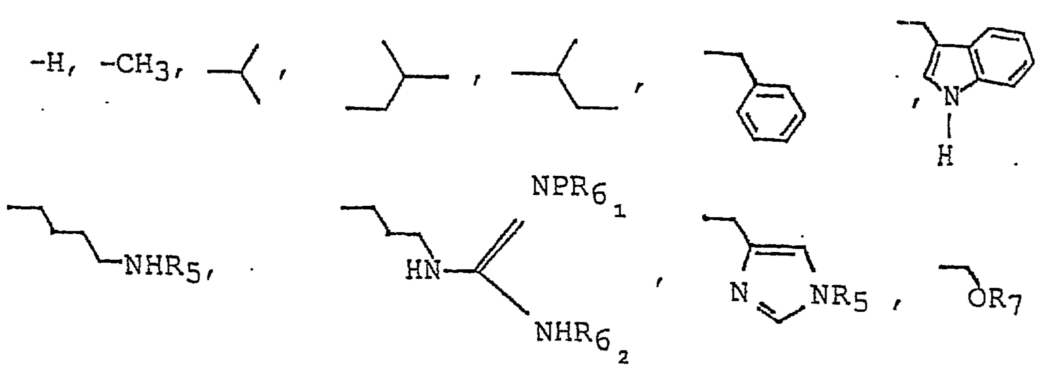


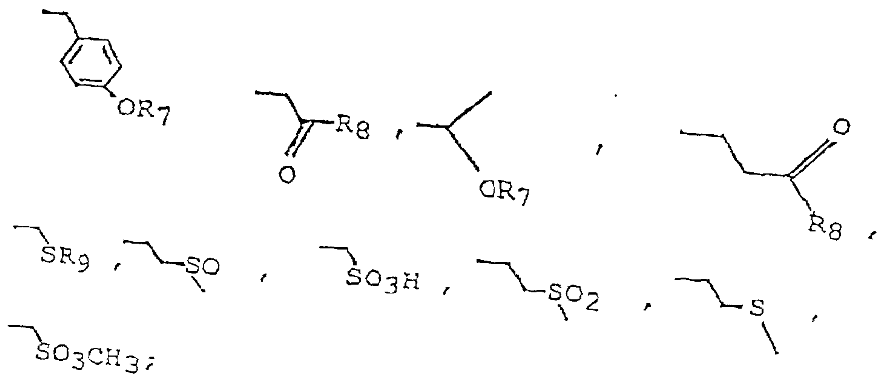
, and



,

- Ar being an aromatic group, substituted aromatic group or a heteroaromatic group,
- a being an integer selected from the group consisting of 0, 1 and 2,
- R being a monovalent group $-(CH_2)_n-CH_3$ or $-(CH_2)_n-Ar$ with n being an integer from 1 to 16,
- R_0, R_1, R_2, R_3 and R_4 being independently selected from the group consisting of:





proline and hydroxyproline may be used at positions 0,2 and 3; and 1 when X is CO,

R_5, R_{6_1} and R_{6_2} each being a monovalent radical

independantly selected from the group consisting of: -H, $-SO_2-CH_3$, $-SO_2-CF_3$, -COH, $-CQCH_3$, -CO-Ar, -CO-R or -CO-NHR wherein R is defined as above, -CONHAr, -COO-tBu and $-COO-CH_2-Ar$, said radical being or not substituted by at least one monovalent group selected in the group consisting of:

$-O-CH_3$, $-CH_3$, $-NO_2$, $-NH_2$, $-NH-CH_3$, $-N(CH_3)_2$, -CO-OH, $-CO-O-CH_3$, $-CO-CH_3$, $-CO-NH_2$, OH, F, Cl, Br and I;

R_7 being a monovalent radical selected from the group consisting of:

-H, -COH, $-CO-CH_3$, NHOH, NHOR, NHR, -CO-R wherein R is defined as above, -CO-Ar and -CO-tBu, said radical being substituted or not by at least one substituent selected from the group consisting of:

$-O-CH_3$, $-CH_3$, $-NO_2$, $-NH_2$, $-NH-CH_3$, $-N(CH_3)_2$, $-CO-OH$, $-CO-O-CH_3$, $-CO-CH_3$, $-CO-NH_2$, OH, F, Cl, Br and I;

R_8 being a monovalent radical selected from the group consisting of: $-OH$, $-NH_2$, $-OCH_3$, $-NHCH_3$, -O-tBu and $-O-CH_2-Ar$, said radical substituted or not by at least one group selected in the group consisting of:

$-O-CH_3$, $-CH_3$, $-NO_2$, $-NH-CH_3$, $-N(CH_3)_2$, $-CO-OH$, $-CO-O-CH_3$, $-CO-CH_3$, $-CO-NH_2$, OH, F, Cl, Br, I;

R_9 being a monovalent radical selected in the group consisting of: -H, -tBu, $-CO-CH_3$, -COAr, -CO-R wherein R is defined as above and -COH, said radicals substituted or not by at least one a monovalent group selected from the group consisting of:

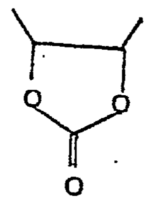
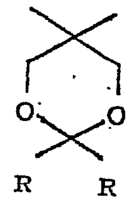
$-O-CH_3$, $-CH_3$, $-NO_2$, $-NH-CH_3$, $-N(CH_3)_2$, $-CO-OH$, $-CO-O-CH_3$, $-CO-CH_3$, $-CO-NH_2$, OH, F, Cl, Br and, I;

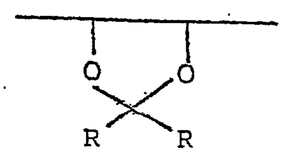
- where Y is a bivalent group $-CH_2-$ or -CO-;
- where Z is a bivalent group -NH- or -O-;
- wherein x, y, z and t are integers each independently selected from the group consisting of 0,1 and 2;
- wherein L is a bivalent radical selected from the group consisting of:

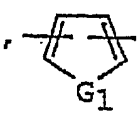
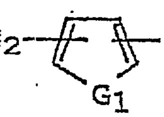
$-(CH_2)_d-A-(CH_2)_j-B-(CH_2)_e-$, d and e being independently an integer from 1 to 5, j being an integer from 0 to 5, when j is 0, A or B is present,

with A and B being independently selected from the group consisting of:

-O-, -NH-, -NR- wherein R is defined as above, -S-, -CO-, -SO-, -CO-O-, -O-CO-, -CO-NH-, -NH-CO-, $-SO_2-NH-$, $-NH-SO_2-$,

 , 

 , -CHOH-,  ,

 , -CH=CH- with the configuration Z or E, -C≡C-,  G_2 and $-G_2-$ 

with the substituent $-G_2-$ in a 1,2, 1,3 or 1,4 position,

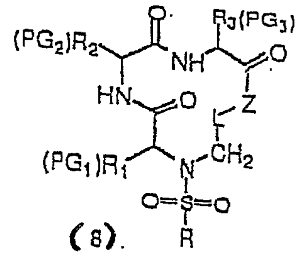
G_1 being selected from the group consisting of:

-O-, -NH-, -NR- wherein R is defined as above, -S-, -CH=CH- with a Z configuration, and -CH=N-; and

G_2 being selected from the group consisting of:

-O-, -NH-, -CO-, -NR- wherein R is defined as above, -CO-O-, -O-CO-, -CO-NH-, -NH-CO-, $-SO_2-NH-$ and $-NH-SO_2-$.

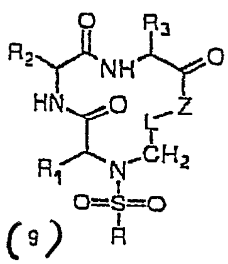
2. A macrocyclic compound according to claim 1, wherein said compound is of formula (8):



(8).

where L, Z, R, R_1, R_2 and R_3 have the same meanings as given in claim 1 and (PG_1), (PG_2) and (PG_3) are protective groups commonly used for orthogonal protections in peptides synthesis.

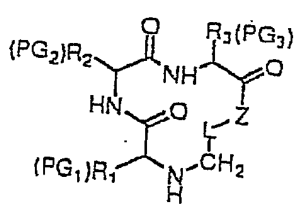
3. A macrocyclic compound according to claim 1, wherein said compound is of the formula (9):



(9)

where L, Z, R, R_1, R_2 and R_3 have the same meanings as given in claim 1.

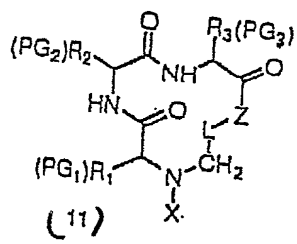
4. A macrocyclic compound according to claim 1, wherein said compound is of the formula (10):



(10)

where L, Z, R_1, R_2 and R_3 have the same meanings as given in claim 1 and (PG_1), (PG_2) and (PG_3) are protective group commonly used for orthogonal protection in peptides synthesis.

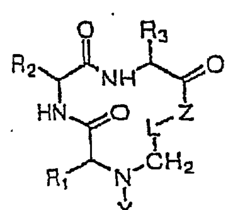
5. A macrocyclic compound according to claim 1, wherein said compound is of the formula (11):



(11)

where L, Z, R_1, R_2 and R_3 have the same meanings as given in claim 1 and wherein (PG_1), (PG_2) and (PG_3) are protective group commonly used for orthogonal protection in peptides synthesis.

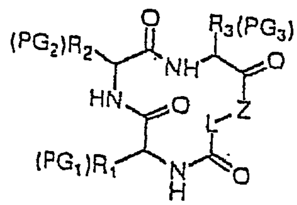
6. A macrocyclic compound according to claim 1, wherein said compound is of the formula (12):



(12)

where L, Z, X, R_1, R_2 and R_3 have the same meanings as given in claim 1.

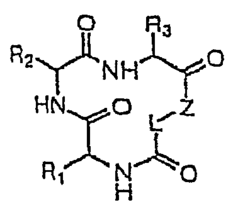
7. A macrocyclic compound according to claim 1, wherein said compound is of the formula (17):



(17)

where X, L, Z, R_1, R_2 and R_3 have the same meanings as given in claim 1 and (PG_1), (PG_2) and (PG_3) are protective group commonly used for orthogonal protection in peptides synthesis.

8. A macrocyclic compound according to claim 1, wherein said compound is of the formula (18):



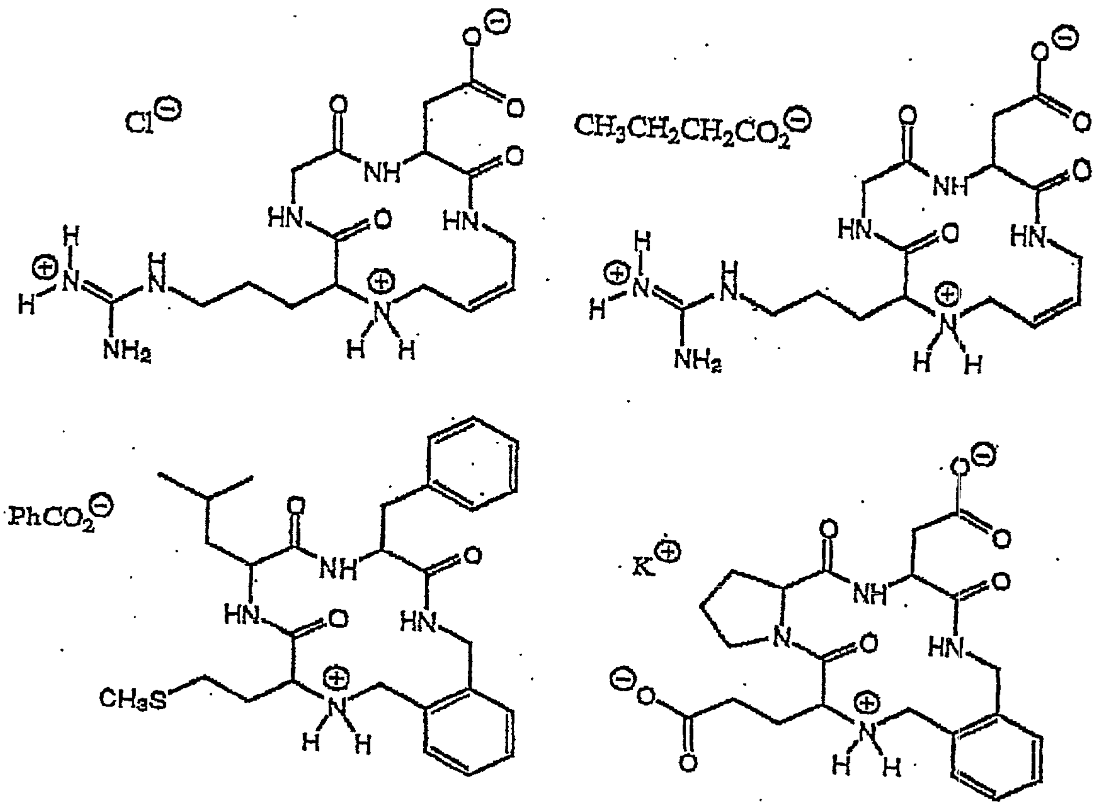
(18)

where L, Z, R_1, R_2 and R_3 have the same meanings as given in claim 1.

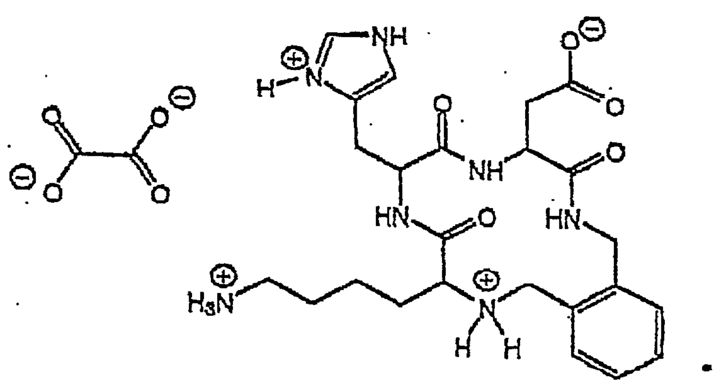
9. A macrocyclic compound according to any one of claims 2, 4, 5 and 7 wherein each of $(PG_1)R_1$, $(PG_2)R_2$, $(PG_3)R_3$,

$(PG_4)R_4$ have independently the same meanings as the radical R_5, R_6, R_7, R_8 or R_9 as defined in claim 1.

10. A macrocyclic compound according to claim 1, selected from the group consisting of:



and

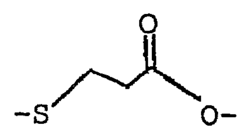
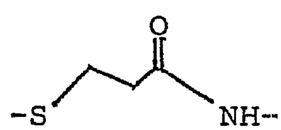


11. A process for preparing a compound of the formula (I) as claimed in claim 1, comprising the steps of:

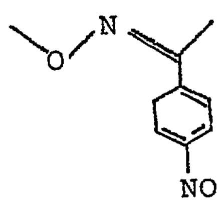
a) preparing by coupling a first building block deriving from natural or synthetic amino-acids, said first building block being of the formula:

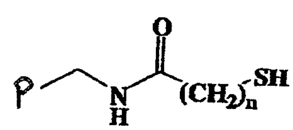
$$\mathrm{H{-}\underset{X}{\underset{|}{N}}{-}\underset{R_1}{\underset{|}{CH}}{-}\underset{O}{\underset{\|}{C}}{-}A{-}B{-}C{-}Sp{-}P}$$

wherein X, R_1, A, B, C are defined as in claim 1,

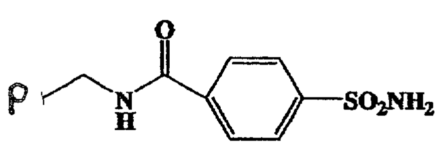
Sp is  or  and

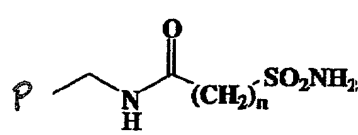
P is $-CH_3$ or $-CH_2$-Ph when the coupling is carried out in liquid phase, and

Sp is 

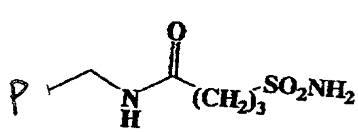


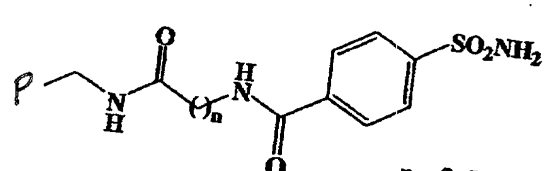
n = 2 (standard), 3, 4, 5, 10





n = 4, 5, 10





n = 3, 5, 9

and P is polystyrene, PEG-polystyrene or polyacrylamide or any suitable resin when the coupling is carried out in solid phase;

b) coupling the first building block prepared in step a) with a second building block hereafter called "tether", of the formula:

$$H-Y-L-Z-PG_Z$$

wherein Y, L and Z are defined as in claim 1 and PG_Z is a protective group; and

c) removing the protection groups PG_Z from the compound obtained in step b); and

d) carrying out a macrocyclization of the unprotected product obtained in step c) and a cleavage if the above mentioned steps (a) and (b) were carried out in a solid phase, in order to obtain the requested compound of the formula (I).

12. A process according to claim 11, wherein when A, B or C is Arg, the process further comprises the steps of

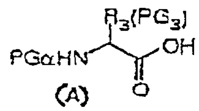
a) utilizing a suitably protected ornithine (Orn) residue as a surrogate for Arg,

b) carrying out a selective deprotection of the protecting on the Orn side chain, and

c) reacting with an appropriately protected guanylating reagent to provide the protected Arg element.

13. A process for preparing a compound of formula (8) as defined in claim 2, comprising the steps of:

a) coupling an amino-acid of the formula A:

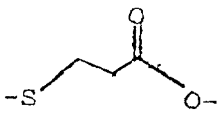
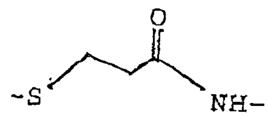


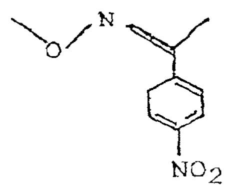
(A)

wherein (PG_α) is an amine protective group and R_3 and PG_3 are defined as in claim 1, either in a solid or a liquid phase, with a compound of the formula:

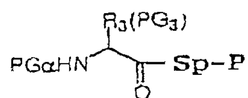
H-Sp-P

wherein, when the coupling is carried out in a liquid phase, Sp is

 or  and P is $-CH_3$ or $-CH_2$-Ph and, when the coupling is carried out in a solid phase, Sp is

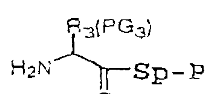
 and P is polystyrene,

in order to obtain a compound of the formula (1)



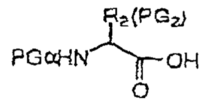
(1)

b) removing the amine protection group PGα from the compound of the formula (1) to obtain the corresponding compound of the formula (2):



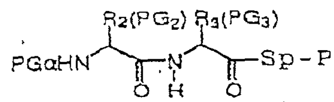
(2)

c) coupling the compound of the formula (2) with another amino-acid of the formula B:



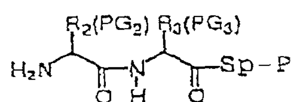
(B)

wherein PGα is defined as above and R_2 and PG_2 are defined as in claim 1, in order to obtain a compound of the formula (3):



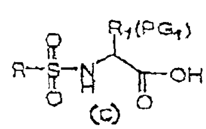
(3)

d) removing the amine protection group PGα from the compound of the formula (3) to obtain the corresponding compound of the formula (4):



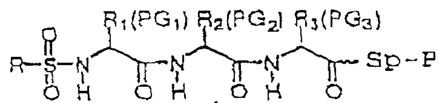
(4)

e) either coupling the compound of the formula (4) with a further amino-acid of the formula (C):



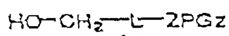
(C)

wherein R is $-(CH_2)_n-CH_3$ or $-(CH_2)_n-Ar$ with n ranging from 1 to 16, in order to obtain a compound of the formula (5):



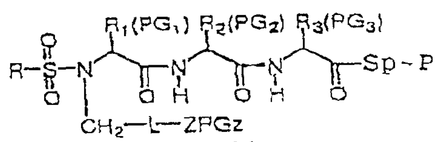
(5) .

and coupling said compound of the formula (5) under Mitsunobu conditions with an alchool of the formula (D):



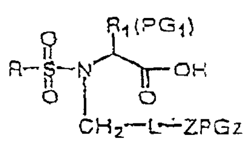
(D)

wherein L and Z are defined as in claim 1 and PG_Z is a protective group, in order to obtain the compound of the formula (6):



(6)

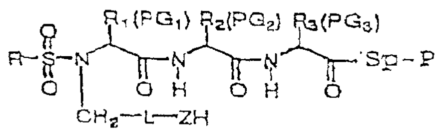
or coupling the compound of the formula (4) with a compound of the formula (E):



(E)

wherein R, L, R_1, PG_1 and PG_2 are defined as above, in order to obtain directly the compound of the formula (6);

f) removing the protective group PG_z from the compound of the formula (6) to obtain the corresponding compound of the formula (7):



(7)

and

g) carrying out a macrocyclisation of the compound of the formula (7) and a cleavage if the coupling steps were carried out in the solid phase, in order to obtain the requested compound of the formula (8).

14. A process for preparing a compound of the formula (9) as defined in claim 3, which comprises the steps defined in claim 13 and a further step which consists in the removal of the protective group PG1, PG2, PG3 of the compound of formula (8) as defined in claim 12 to yield the requested compound of formula (9).

15. A process for preparing a compound of formula (10) as defined in claim 4, which comprises the steps defined in claim 13 and a further step which consists in the cleaving

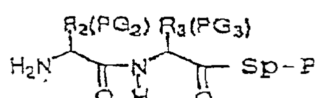
of the sulfonamide portion of the compound of formula (8) to yield the requested compound of formula (10) with a free amine group.

16. A process for preparing a compound of formula (11) as defined in claim 5, which comprises the steps defined in claim 15 and a further step which consists in coupling the free amine of the compound of formula (10) with an acid of formula HX, wherein X has the meaning given in claim 1, to yield the requested compound of formula (11).

17. A process for preparing a compound of formula (12) as defined in claim 6 which comprises the steps defined in claim 14 and a further step which consists in cleaving the orthogonal protecting groups PG1, PG2 and PG3 of the compound of formula (II) to yield the requested compound of formula (12).

18. A process for preparing a compound of formula (17) as defined in claim 7, comprising the steps of:

a) coupling an amine of formula (4):

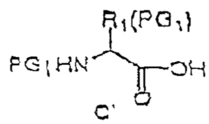


(4)

wherein $R_2(PG_2)$, $R_3(PG_3)$ are protective groups commonly used for orthogonal protection in peptides synthesis, A has the same meaning as given in claim 1, and P is $-CH_3$ or $-CH_2$-Ph and,

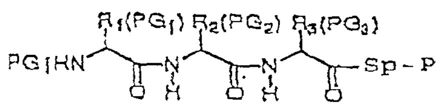
when the coupling is carried out in solid phase, P may also be polystyrene,

with the amino-acid of formula (C'):



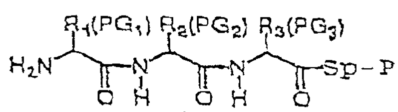
C'

wherein $R_1(PG_1)$ is a protective group commonly used for orthogonal protection in peptides synthesis and wherein PGα is an amine protective group, in order to obtain a compound of formula (13):



13

b) removing the amino protecting group (PGα) from the compound of formula (13) to obtain the corresponding compound of formula (14):



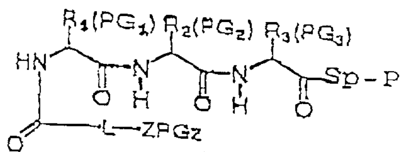
(14)

c) coupling the compound of formula (14) with an hydroxy-acid (Z=O) or with an amino-acid (Z=NH) of formula (D'):

$HOOC-L-ZPG_2$

D'

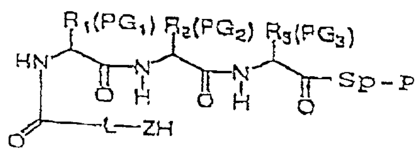
wherein L has the same meaning as in claim 1 and PG_Z is a protective group, to obtain the compound of formula (15):



(15)

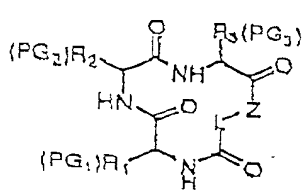
wherein PG_Z is a protective group,

d) removing the terminal alcohol (Z=O) or amine (Z=H) protecting group (PG_Z) from the compound of formula (15) to obtain the corresponding alcohol or amine of the formula (16):



(16)

e) carrying out a macrocyclisation of the compound and a cleaving of formula (16) all at once the compound of formula (16) to obtain the requested compound of formula (17):



(17)

19. A process for preparing a compound of formula (18) as defined in claim 8 which comprises the step defined in claim 17 and a further step of removing the orthogonal protection (PG1), (PG2), (PG3) and (PG4), when a fourth amino-acid is introduced, from the compound of formula (17) to yield to the requested compound of formula (18).

20. A process according to any one of claims 11, 13, 16, 17 and 18, wherein each of $(PG_1)R_1$, $(PG_2)R_2$, $(PG_3)R_3$, $(PG_4)R_4$ and (PGz)Z have independently the same meanings as the radical R_5, R_6, R_7, R_8 or R_9 as defined in claim 1.

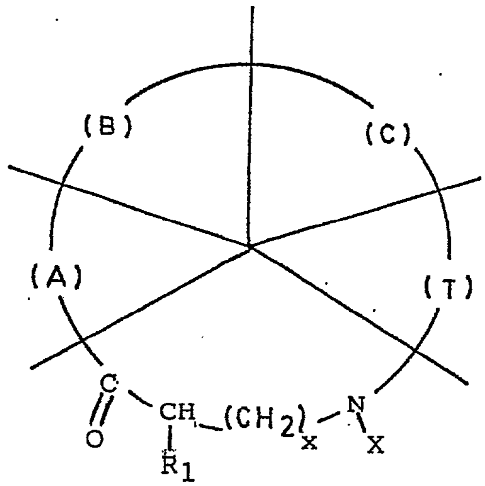
21. A process according to claim 19, wherein at least one of PG_1, PG_2, PG_3, PG_4 and PG_z is a carbamate or a trityl group.

22. A process according to claim 21, wherein the carbamate is selected from the group consisting of Boc, Fmoc and Ddz.

23. A process according to claim 21, wherein the trityl is selected from the group consisting of Trt and Mmt.

ABSTRACT

A library of macrocyclic compounds of the formula (I)



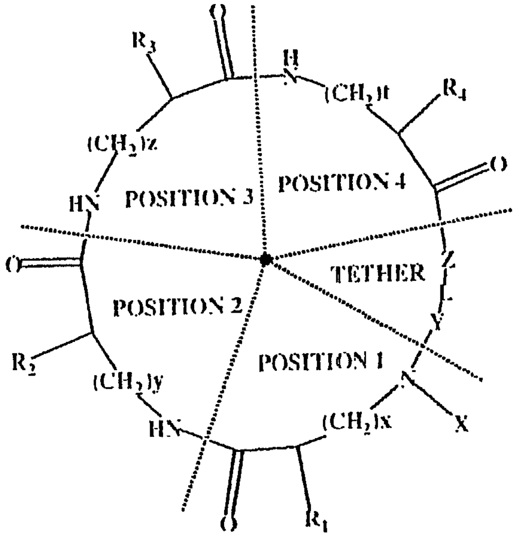
(I)

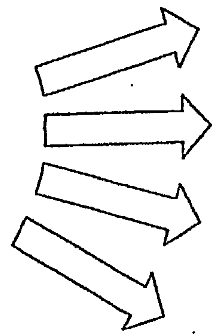
- where part (A) is a $-\underset{\underset{O}{\|}}{C}-\underset{\underset{R_2}{|}}{CH}-(CH_2)_y-NH-$ bivalent radical,

  a $-(CH_2)_y-$ bivalent radical or a covalent bond;
- where part (B) is a $-\underset{\underset{O}{\|}}{C}-\underset{\underset{R_3}{|}}{CH}-(CH_2)_z-NH-$ bivalent radical,

  a $-(CH_2)_z-$ bivalent radical, or a covalent bond;
- where part (C) is a $-\underset{\underset{O}{\|}}{C}-\underset{\underset{R_4}{|}}{CH}-(CH_2)_t-NH-$ bivalent radical,

  a $-(CH2)_t-$ bivalent radical, or a covalent bond; and
- where part (T) is a $-Y-L-Z-$ radical wherein Y is CH_2 or CO, Z is NH or O and L is a bivalent radical. These compounds are useful for carrying out screening assays or as intermediates for the synthesis of other compounds of pharmaceutical interest. A process for their preparation of these compounds in a combinatorial manner, is also disclosed.



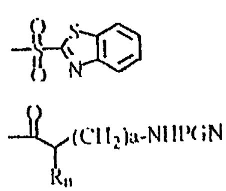
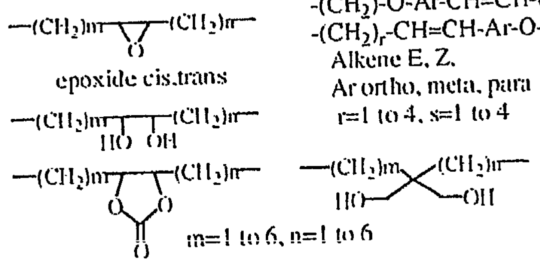
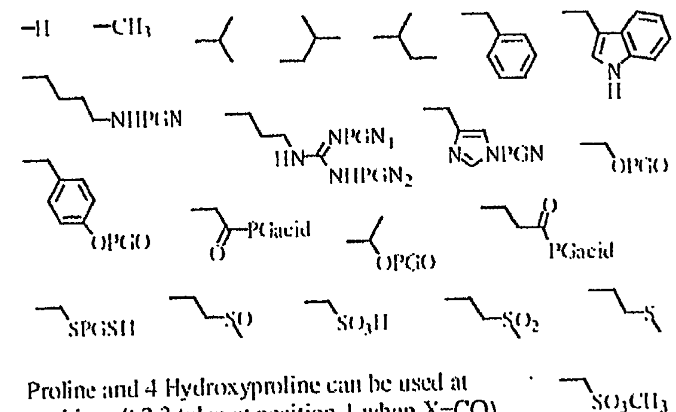


FAMILY 1: see table

FAMILY 2: like family 1 with position 4 empty

FAMILY 3: like family 1 with positions 3 and 4 empty

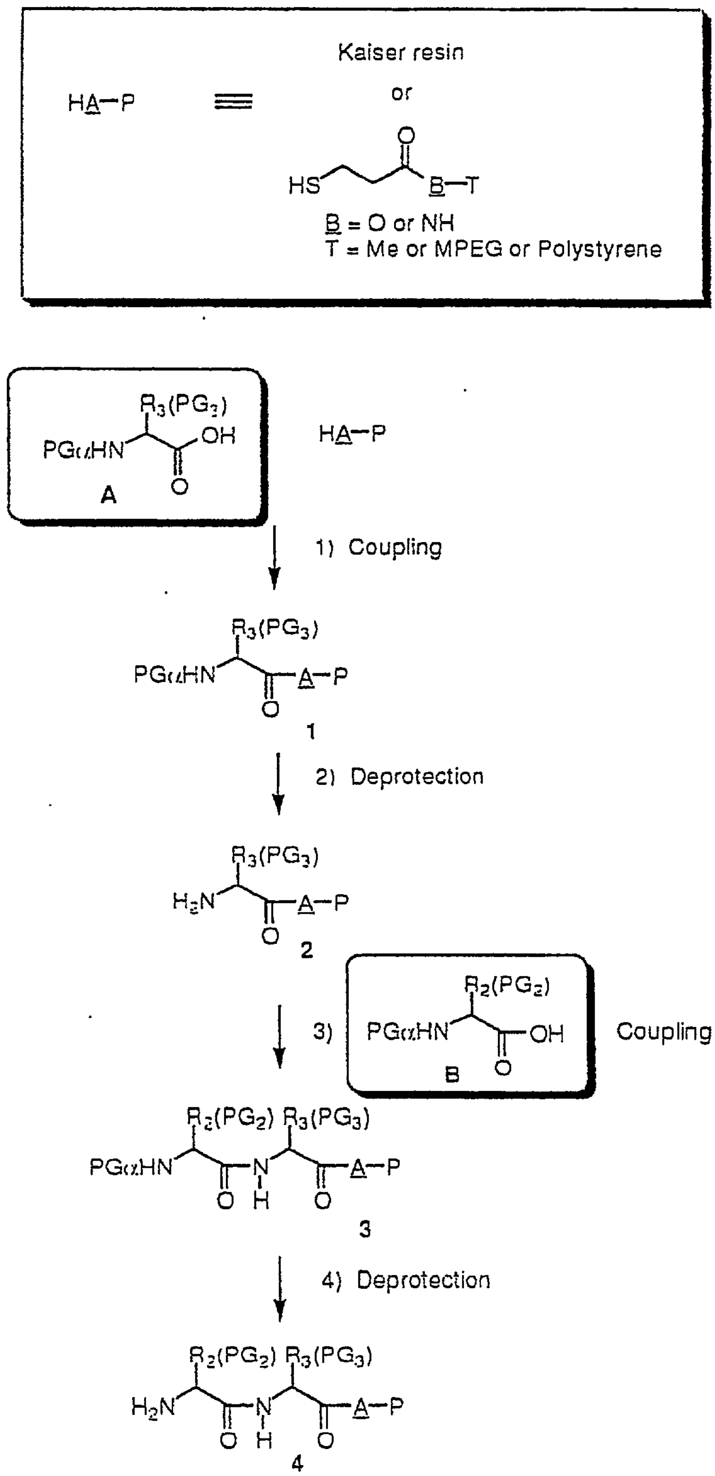
FAMILY 4: like family 1 with positions 2, 3 and 4 empty

X	Y	Z	L	x y z t (a)	R_0 R_1 R_2 R_3 R_4
$-SO_2-Ar$ $-SO_2-CH_3$ $-SO_2-CF_3$ -H -COH $-CO-CH_3$ -CO-Ar -CO-NHR -CO-NHAr -CO-O-tBu -CO-O-Bn 	$-CH_2-$ -CO-	-NH- -O-	$-(CH_2)_i-$ i=1 to 9 $-(CH_2)_m-Ar-(CH_2)_n-$ $-(CH_2)_m-O-Ar-(CH_2)_n$ $-(CH_2)_m-Ar-O-(CH_2)_n-$ Ar ortho, meta, para $-(CH_2)_m-CH{=}CH-(CH_2)_n-$ Alkene E,Z $-(CH_2)_m-C{\equiv}C-(CH_2)_n-$ $-(CH_2)_r-Ar-CH{=}CH-(CH_2)_s-$ $-(CH_2)_r-CH{=}CH-Ar-(CH_2)_s-$ $-(CH_2)-O-Ar-CH{=}CH-(CH_2)_s-$ $-(CH_2)_r-CH{=}CH-Ar-O-(CH_2)_s-$ Alkene E, Z. Ar ortho, meta, para r=1 to 4, s=1 to 4  epoxide cis,trans m=1 to 6, n=1 to 6	0 1 2	 Proline and 4 Hydroxyproline can be used at positions 0,2,3 (also at position 1 when X=CO)
					PGO= -H, -COH, $-CO-CH_3$, -CO-Ar, CO-tBu PGacid= -OH, $-NH_2$, $-OCH_3$, $-NHCH_3$, -O-tBu, -O-Bn

PGN= -H, $-SO_2-CH_3$, $-SO_2-CF_3$, -COH, $-CO-CH_3$, -CO-Ar, -CO-NHR, -CO-NHAR, -CO-O-tBu, -CO-O-Bn, -CO-O-R PGS= -H, -tBu, $-CO-CH_3$, -CO-Ar, -COH

Ar, alkenes and CH_2s in AA (Amino-Acid), PGN, PGO, PGacid and PGS can bear groups amongst : $-O-CH_3$, $-CH_3$, $-NO_2$, $-NH_2$, $-NH-CH_3$, $-N(CH_3)_2$, -CO-OH, $-CO-O-CH_3$, $-CO-CH_3$, $-CO-NH_2$, OH, F, Cl, Br, I.

FIGURE 1



A = Sp

FIGURE 2a

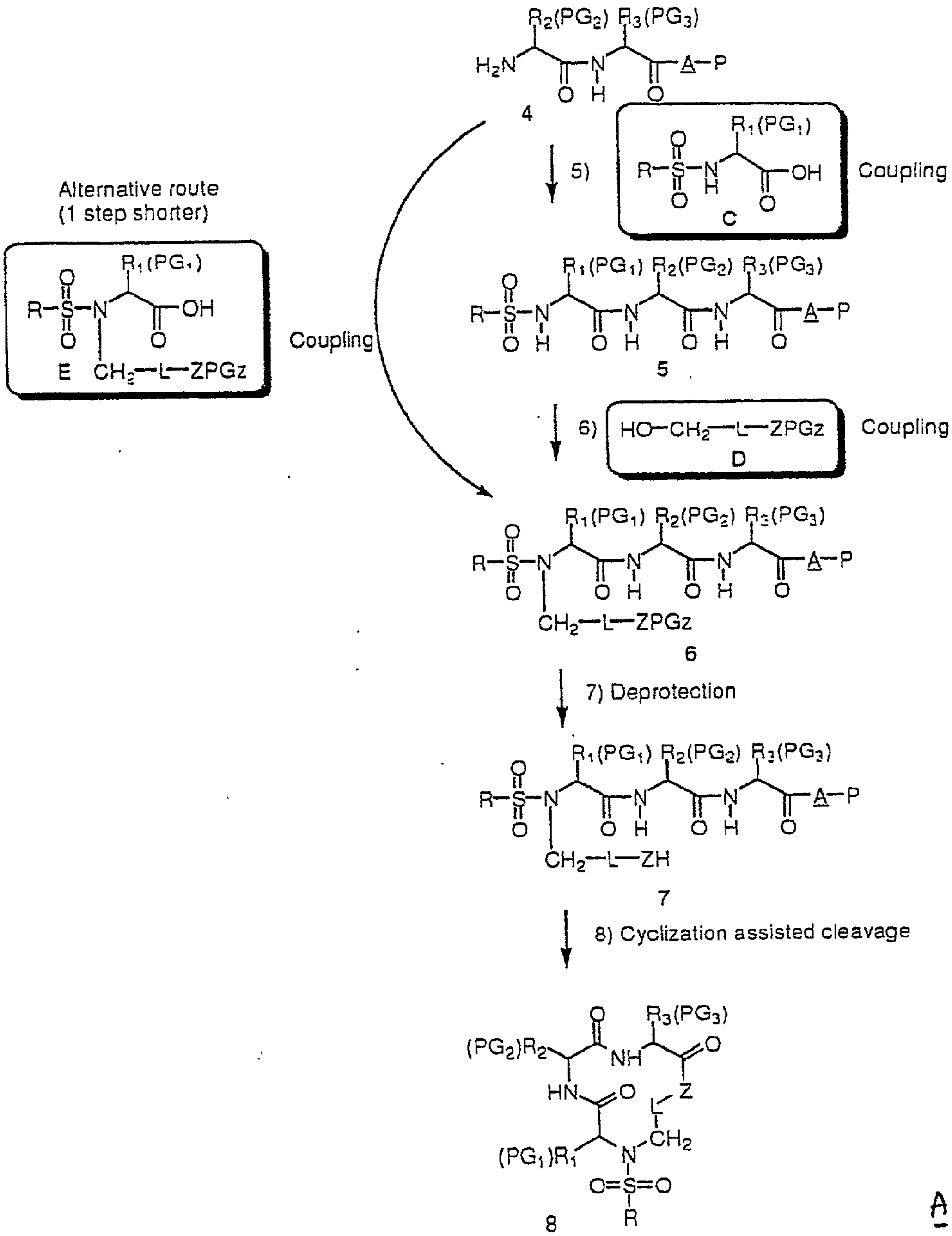
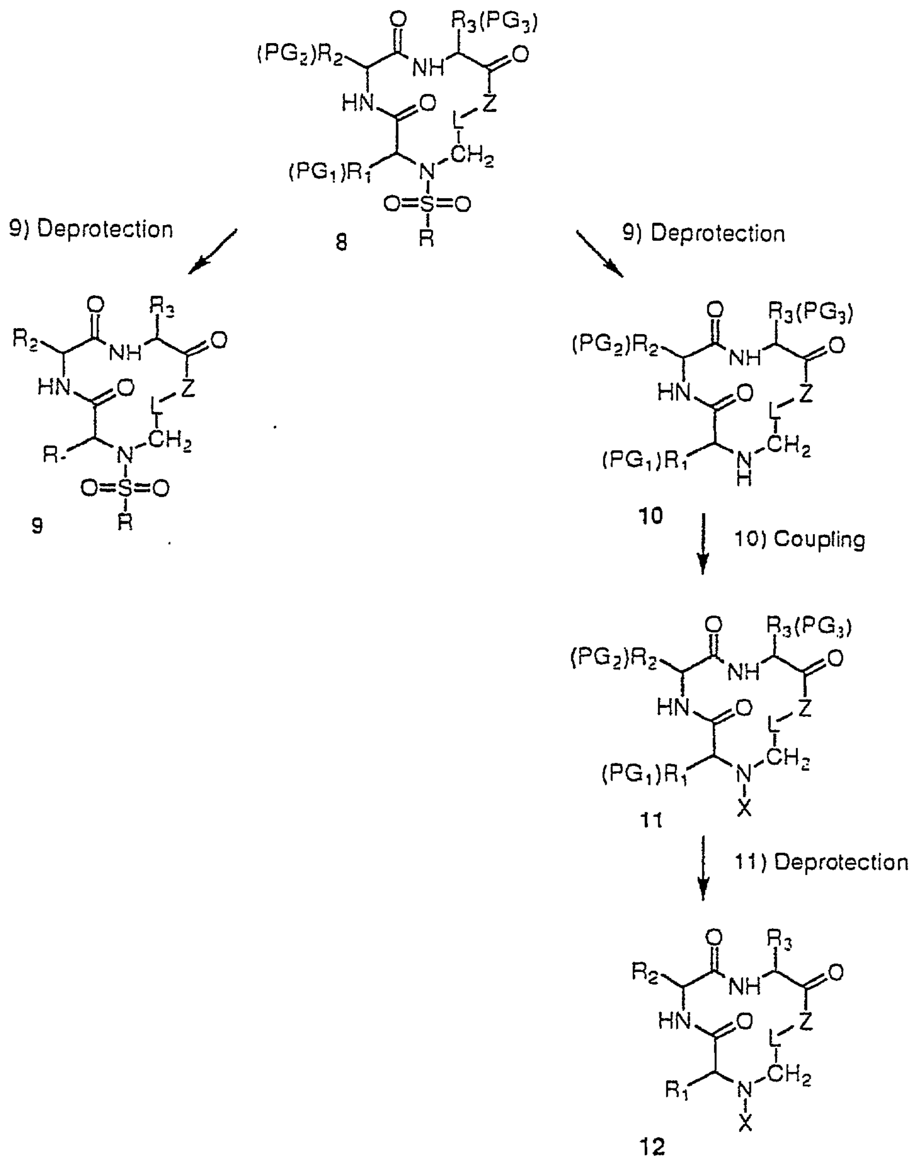


FIGURE 2b

A = Sp



9) Deprotection
9) Deprotection
10) Coupling
11) Deprotection

FIGURE 3

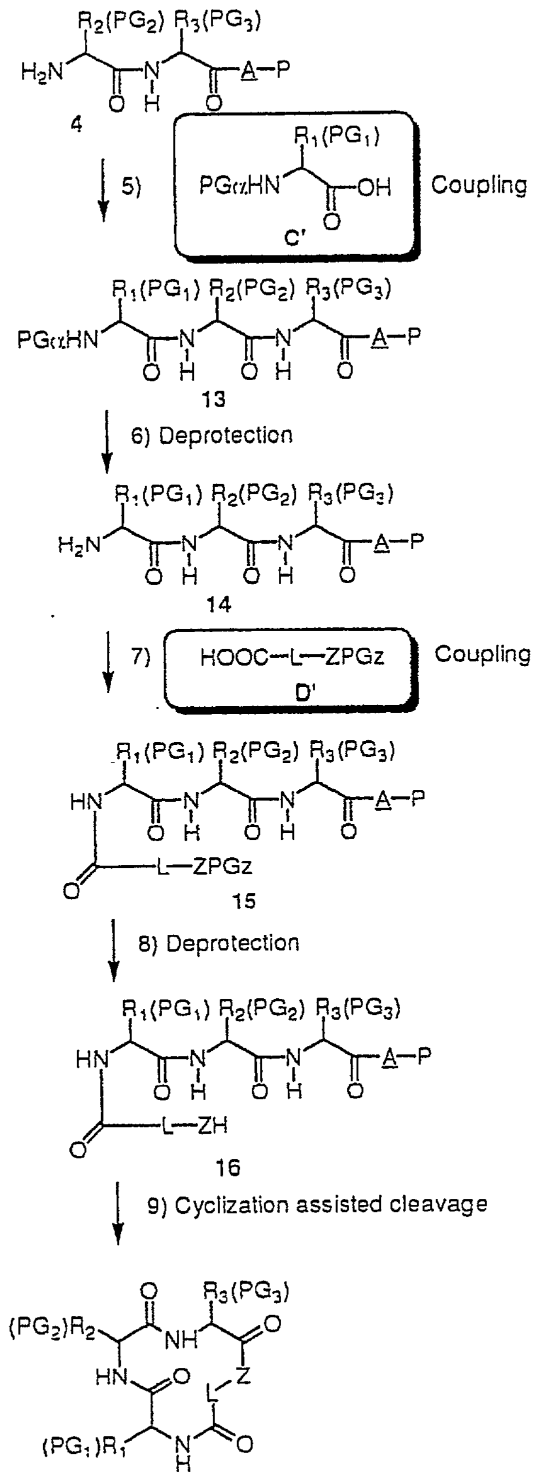


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FIGURE 4a

$\underline{A} = Sp$

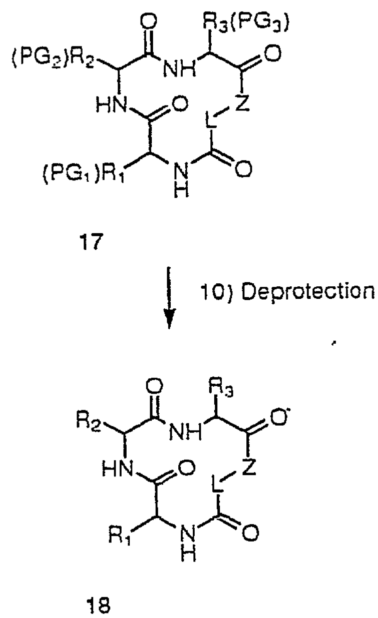

FIGURE 4b

Docket No.: **6670/0H748**

DECLARATION
AND POWER OF ATTORNEY
Original Application

As a below named inventor, I declare that the information given herein is true, that I believe that I am the original, first and sole inventor if only one name is listed at 1 below, or a joint inventor if plural inventors are named below, of the invention entitled:

COMBINATORIAL SYNTHESIS OF LIBRARIES OF MACROCYCLIC COMPOUNDS USEFUL IN DRUG DISCOVERY

which is described and claimed in:

[**X**] the attached specification or

[] the specification in application Serial No. , filed
(for declaration not accompanying appl.)

that I do not know and do not believe that the same was ever known or used in the United States of America before my or our invention thereof or patented or described in any printed publication in any country before my or our invention thereof, or more than one year prior to this application, or in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to this application, that I acknowledge my duty to disclose information of which I am aware which is material to patentability in accordance with 37 CFR §1.56. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I hereby claim the priority benefits under 35 U.S.C. §119 of any application(s) for patent or inventor's certificate listed below. All foreign applications for patent or inventor's certificate on this invention filed by me or my legal representatives or assigns prior to the application(s) of which priority is claimed are also identified below.

PRIOR APPLICATION(S), IF ANY, OF WHICH PRIORITY IS CLAIMED

COUNTRY	APPLICATION NO.	DATE OF FILING
CANADA	2,284,459	OCT. 4, 1999

ALL FOREIGN APPLICATIONS, IF ANY, FILED PRIOR TO THE APPLICATION(S) OF WHICH PRIORITY IS CLAIMED

COUNTRY	APPLICATION NO.	DATE OF FILING

POWER OF ATTORNEY:

As a named inventor, I hereby appoint the following attorney(s) and/or agents(s) to prosecute this application and transact all business in the Patent and Trademark office connected therewith: Gordon D. Coplein #19,165, William F. Dudine, Jr. #20,569, Michael J. Sweedler #19,937, S. Peter Ludwig #25,351, Paul Fields #20,298, Marc S. Gross #19,614, Joseph B. Lerch #26,936, Melvin C. Garner #26,272, Ethan Horwitz #27,646, Beverly B. Goodwin #28,417, Adda C. Gogoris #29,714, Martin E. Goldstein #20,869, Bert J. Lewen #19,407, Henry Sternberg #22,408, Peter C. Schechter #31,662, Robert Schaffer #31,194, Robert C. Sullivan, Jr. #30,499, Ira J. Levy #35,587, Joseph R. Robinson #33,448, Scott G. Lindvall #40,325

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I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 1: ______________________ DATED: ____________
Pierre DESLONGCHAMPS

SIGNATURE OF INVENTOR 2: ______________________ DATED: ____________
Yves DORY

SIGNATURE OF INVENTOR 3: ______________________ DATED: ____________
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SIGNATURE OF INVENTOR 4: ______________________ DATED: ____________
Luc OUELLET

SIGNATURE OF INVENTOR 5: ______________________ DATED: ____________
Ruoxi LAN